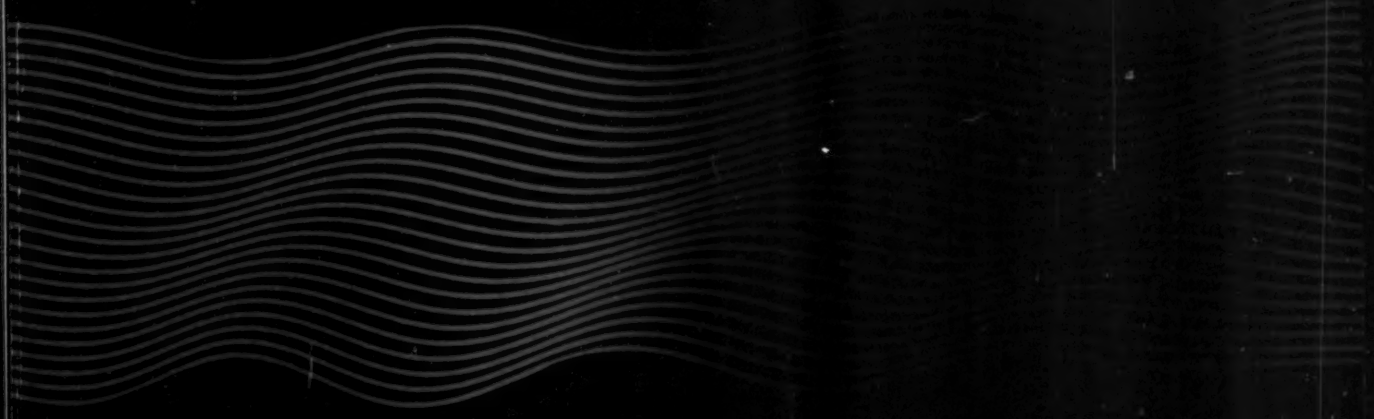
National Research Council Canada

Conseil national de recherches Canada

NRC · CNRC

# Canadian Journal of Fisheries and Aquatic Sciences

# Journal canadien des sciences halieutiques et aquatiques



The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79. The *Journal* is published by the National Research Council of Canada in cooperation with the Department of Fisheries and Oceans.

The *Journal* is issued monthly to form annual volumes of twelve numbers plus Supplements.

**Editorial Policy**

The *Journal* publishes original research articles, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

**Guides**

A guide for authors appears in the first issue of each volume and is available free from the *Journal*.

**Reprints**

Reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent back with the galley proofs. Reprint phone enquiries: Eileen Fink at (613) 993-0151.

**Advertisements**

Advertisement rates and information can be obtained from Hoda Jabbour at (613) 993-9085. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the *Journal* or the National Research Council of Canada.

**Microfilm**

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

The publisher grants permission to individuals who wish to quote short excerpts and reproduce figures, tables, or photographs from articles in this journal, provided that the source of such material is fully acknowledged. As a courtesy the consent of authors of such material should be obtained.

©National Research Council of Canada, 1993. World rights reserved.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by the National Research Council of Canada for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of $3.00 per copy is paid directly to CCC, 21 Congress Street, Salem, MA 01970, U.S.A. 0008-4212/92 $3.00 + 0.00.

The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher.

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979. Le *Journal* est publié par le Conseil national de recherches du Canada en collaboration avec le ministère des Pêches et des Océans.

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros, et offre en plus jusqu'à deux suppléments par année.

**Politique de rédaction**

Le *Journal* publie des articles fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, b) des arbitres possibles et c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

**Guides**

Le guide des auteurs paraît dans la première livraison de chaque volume et est offert gratuitement par le *Journal*.

**Tirés à part**

Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves de galée et doit être retourné avec celles-ci. Renseignements supplémentaires : Eileen Fink au (613) 993-0151.

**Annonces publicitaires**

Pour toute information concernant les annonces publicitaires ou leur coût, s'adresser à Hoda Jabbour au (613) 993-9085. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par le *Journal* ou le Conseil national de recherches du Canada.

**Microfilms**

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

L'éditeur accorde aux particuliers la permission de citer de brefs extraits et de reproduire des figures, des tableaux ou des photos dans les articles du *Journal*, à la condition que la source soit indiquée explicitement. Dans ce cas, il convient, par courtoisi, d'obtenir le consentement des auteurs.

©Conseil national de recherches du Canada 1993. Tous droits réservés.

Le Conseil national de recherches du Canada autorise les bibliothèques et autres utilisateurs inscrits au Copyright Clearance Center (CCC) Transactional Reporting Service à photocopier les documents destinés à un usage interne ou personnel, ou à l'usage interne ou personnel de clients particuliers, à la condition que le tarif de base de 3 $ par copie soit payé directement au CCC, 21 Congress Street, Salem, MA 01970, U.S.A. 0008-4212/92 3,00 $ + 0,00.

La présente permission ne s'applique pas à d'autres genres de reproduction, notamment la reproduction en vue d'une distribution générale, à des fins de publicité ou de promotion, pour la création de nouveaux travaux collectifs, ou pour la revente. Dans ces cas, il faut prendre les dispositions qui s'imposent en communiquant avec l'éditeur.

# Canadian Journal of Fisheries and Aquatic Sciences

# Journal canadien des sciences halieutiques et aquatiques

**Volume 50, No. 11, November 1993** **Volume 50, nº 11, novembre 1993**

D.G. Cook — *Editor/Rédacteur*
M.M. Milne — *Assistant to the Editor/Adjointe au rédacteur*
P.E.K. Symons — *Consulting Editor/Rédacteur-conseil (Victoria, B.C.)*
N. Moir — *Consulting Editor/Rédactrice-conseil (Nepean, Ont.)*

**Editorial Board/Conseil de rédaction**

L.M. Dickie — *DFO/MPO, Dartmouth (Advisor/Conseiller)*
J.D. Neilson — *CFRAMP, St. Vincent (Associate Editor/Rédacteur associé)*
L.S. Parsons — *DFO/MPO, Ottawa (Advisor/Conseiller)*
R.M. Peterman — *Simon Fraser University (Advisor/Conseiller)*
J.C. Roff — *University of Guelph (Advisor/Conseiller)*
P. Schwinghamer — *DFO/MPO, St. John's (Deputy Editor/Rédacteur adjoint)*
N.H.F. Watson — *DFO/MPO, Halifax (Associate Editor/Rédacteur associé)*

**Research Journals/Revues scientifiques**

Bruce P. Dancik — *Editor-in-chief/Directeur général*
Iain E. P. Taylor — *Assistant Editor-in-chief/Directeur général adjoint*

**Publishing Department/Service de publication**

Aldyth Holmes — *Director/Directrice*
Joan E. Hill — *Operations Manager/Responsable des opérations*
Christine Shugar — *Head of Publications/Chef des publications*
Peter M. Burke — *Publication Officer/Agent de publication*

**Editorial Office:**

Research Journals
National Research Council of Canada
Ottawa, Ontario, Canada
K1A 0R6
Telephone (613) 993-2209
FAX (613) 952-7656
E-mail: research.journals@nrc.ca

**Bureau de rédaction :**

Revues scientifiques
Conseil national de recherches du Canada
Ottawa (Ontario), Canada
K1A 0R6
Téléphone (613) 993-2209
Télécopieur (613) 952-7656
Courrier électronique : research.journals@nrc.ca

*Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Biological & Agricultural Index, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, and Science Citation Index*

# Physiological and Taxonomic Separation of Two Dreissenid Mussels in the Laurentian Great Lakes

S. Domm, R.W. McCauley,[1] and E. Kott

*Department of Biology, Wilfrid Laurier University, Waterloo, ON N2L 3C5, Canada*

and J.D. Ackerman

*Department of Mechanical Engineering, University of Toronto, Toronto, ON M5S 1A4, Canada*

Domm, S., R.W. McCauley, E. Kott, and J.D. Ackerman. 1993. Physiological and taxonomic separation of two dreissenid mussels in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 50: 2294–2297.

Physiological techniques were used to separate two related *Dreissena* species initially established by electrophoretic and morphological characteristics (May and Marsden. 1992. Can. J. Fish. Aquat. Sci. 49: 1501–1506). Samples of zebra mussels (*Dreissena polymorpha*) and "quagga" mussels (provisionally either *Dreissena polymorpha andrusovi* or *Dreissena rostriformis bugensis*) of the same size growing side by side were collected in Lake Erie, thereby ensuring that they had identical thermal histories. Upper lethal temperatures of zebra mussels were significantly higher than those of quagga mussels. The critical thermal maxima of zebra mussels acclimatized at 20°C were half a degree higher than those of quagga mussels. The average survival times of zebra mussels held at a constant lethal temperature were also significantly longer. These results indicate that in addition to differences in allozyme loci and shell morphology, these two dreissenids may be distinguished by important physiological differences in their thermal resistance; moreover, there may be important ecological differences associated with the different species of dreissenids in North America.

Des techniques physiologiques ont été utilisées pour séparer deux espèces apparentées de *Dreissena* reconnues initialement par leurs caractères électrophorétiques et morphologiques (May et Marsden. 1992. J. can. sci. halieut. aquat. 49: 1501–1506). Des spécimens de moules zébrées (*Dreissena polymorpha*) et de moules « quagga » (provisoirement *Dreissena polymorpha andrusovi* ou *Dreissena rostriformis bugensis*) de même taille se développant côte à côte ont été prélevés dans le lac Érié, ce qui a permis de s'assurer qu'elles avaient été exposées aux mêmes conditions thermiques. Les températures mortelles supérieures des moules zébrées étaient significativement plus élevées que celles des moules quagga. La température maximale critique des moules zébrées acclimatées à 20°C était un demi-degré plus élevée que celle des moules quaggas. Le temps de survie moyen des moules zébrées maintenues à une température létale constante était aussi significativement plus long. Ces résultats montrent qu'en plus de différences des loci des allozymes et de la morphologie de la coquille, ces deux espèces de dreissénidés peuvent être distinguées par des différences physiologiques importantes au niveau de leur résistance thermique; de plus, il peut y avoir des différences écologiques importantes associées aux différentes espèces de dreissénidés en Amérique du Nord.

*Received February 11, 1993*
*Accepted June 23, 1993*
*(JB798)*

*Reçu le 11 février 1993*
*Accepté le 23 juin 1993*

The introduction of zebra mussels (*Dreissena polymorpha* (Pallas)) into North American waters has been well documented (Hebert et al. 1989; Nalepa and Schloesser 1993). In 1991, a second form of dreissenid, which was given the working name "quagga" after a relative of the (equine) zebra, was identified in Lake Ontario where it was found to cooccur with zebra mussels (May and Marsden 1992). Quagga mussels were distinguished on the basis of shell morphology and electrophoresis. Ludyanskiy (1993) tentatively identified this second form as either a subspecies (*Dreissena polymorpha andrusovi*) or congener (*Dreissena rostriformis bugensis*), both of which were hitherto found only in the Black Sea – Caspian Sea region. Conversely, zebra mussels (*Dreissena polymorpha polymorpha*) are found throughout Europe. Although the North American introduction of quagga mussels is thought to have occurred in 1989, there is evidence of their presence as early as 1987 (e.g., shells lengths >29 mm collected in 1990, J.D. Ackerman).

Some uncertainty remains as to the taxonomic and ecological distinction of these two "zebra mussels," and therefore, a separate physiologically based method of discrimination was sought. The upper lethal temperature method is one such approach that has been used with traditional morphological characters to separate closely related taxa (Fry 1956). For valid comparisons, it is important that the thermal history (i.e., acclimation) of both groups be identical so that phenotypic effects can be distinguished from genotypic ones. Moreover, nonthermal factors such as season, photoperiod, and other physical conditions must also be similar (Beitinger and McCauley 1990). This approach has long been used to separate fish taxa (Hart 1952; McCauley 1958; Hirshfield et al. 1980; Kott et al. 1980) and is applied, in the present case, to the two dreissenids in the Great Lakes.

[1]Author to whom correspondence should be addressed.

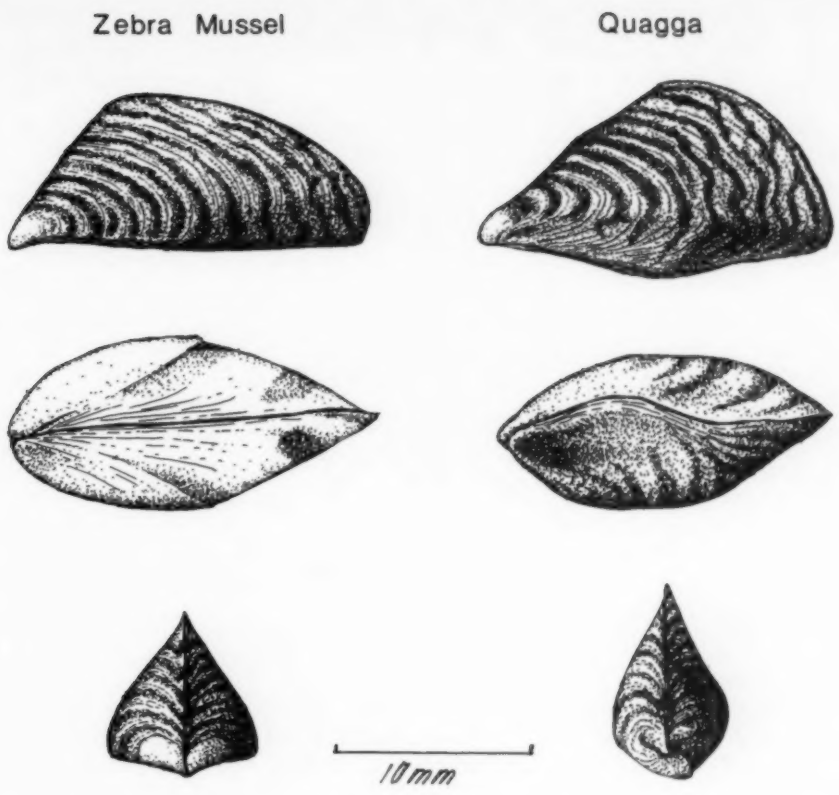



FIG. 1. Lateral, ventral, and anterior views of zebra and quagga mussels showing morphological differences. Note flattened ventral surface of zebra mussel and extension of right valve over midline in quagga mussel.

## Materials and Methods

### Source of Mussels

Specimens of zebra and quagga mussels ranging in size from 8 to 12 mm were collected in August and November 1992 at two sites on the northeastern shore of Lake Erie – Port Dover, Ontario, and Ontario Hydro's Nanticoke Thermal Generating Station (NTGS) in Nanticoke, Ontario. Both forms were found in similar numbers attached to rocks in Port Dover and to concrete plates in the forebay (influent channel) of NTGS, 6 km to the east. The mussels were removed from substrates and transported within 2 h to the laboratory where they were maintained in aerated lake water at room temperature (20–23°C). The August samples were held at room temperature for 5 d prior to testing during which time they were fed a suspension of *Chlorella*. The November samples were collected at a lake water temperature of 4°C and held overnight at 20°C before being transferred to a constant lethal temperature bath.

### Discrimination between Zebra and Quagga Mussels

Although electrophoresis may be used for distinguishing these dreissenids (May and Marsden 1992), easily observed morphological characters are more useful for physiological study. Zebra mussels have a distinct angle or carina between the ventral and dorsal surface, when viewed laterally or from the front, resulting in a flattened ventral surface, whereas the quagga mussel has a smoothly rounded transition from dorsal to ventral surfaces and therefore a more rounded ventral surface (Fig. 1). Unfortunately, discrimination, especially of small individuals, is not always possible based on this character alone. Herein we report an undescribed character in quagga mussels that is easily observed even in small individuals. In quagga mussels viewed from the front or from the ventral side, the valves are distinctly asymmetrical and do not meet along a straight midventral line. Instead, anteriorly the right valve curves over the midventral line (Fig. 1). Conversely, the zebra mussel shells are bilaterally symmetrical and join together in the midventral plane (Fig. 1). Voucher specimens of zebra mussels (ROMIZ M8682) and quagga mussels (ROMIZ M8683) have been deposited in the Department of Invertebrate Zoology of the Royal Ontario Museum in Toronto.

### Determination of Critical Thermal Maximum

The critical thermal maximum (CTmax) of poikilothermic animals is determined by slowly increasing environmental temperatures (from their acclimation level) at a constant rate until the temperature is reached at which the individual is unable to escape the heat stress by locomotory behaviour (Cowles and Bogert 1944; Becker and Genoway 1979; McCauley and Kilgour 1986). This procedure was modified in the present case

TABLE 1. CTmax (°C) of zebra (ZM) and quagga mussels (QM) acclimated to 20°C. Nine replicate runs, in which three ZM and three QM were examined, are presented.

| Run | ZM | QM | Run | ZM | QM | Run | ZM | QM |
|---|---|---|---|---|---|---|---|---|
| 1 | 36.8 | 35.0 | 4 | 36.4 | 36.0 | 7 | 36.0 | 35.8 |
| | 36.8 | 36.5 | | 37.0 | 36.0 | | 36.4 | 35.8 |
| | 37.0 | 36.8 | | 37.0 | 36.4 | | 36.8 | 36.0 |
| 2 | 36.3 | 35.1 | 5 | 36.2 | 35.5 | 8 | 36.8 | 36.8 |
| | 36.8 | 36.0 | | 37.1 | 36.0 | | 37.2 | 37.0 |
| | 37.0 | 36.3 | | 37.2 | 36.8 | | 38.0 | 37.0 |
| 3 | 36.0 | 36.8 | 6 | 37.0 | 36.0 | 9 | 36.9 | 37.2 |
| | 37.1 | 37.0 | | 37.0 | 36.8 | | 37.8 | 37.2 |
| | 37.8 | 37.1 | | 37.0 | 37.0 | | 39.0 | 37.8 |

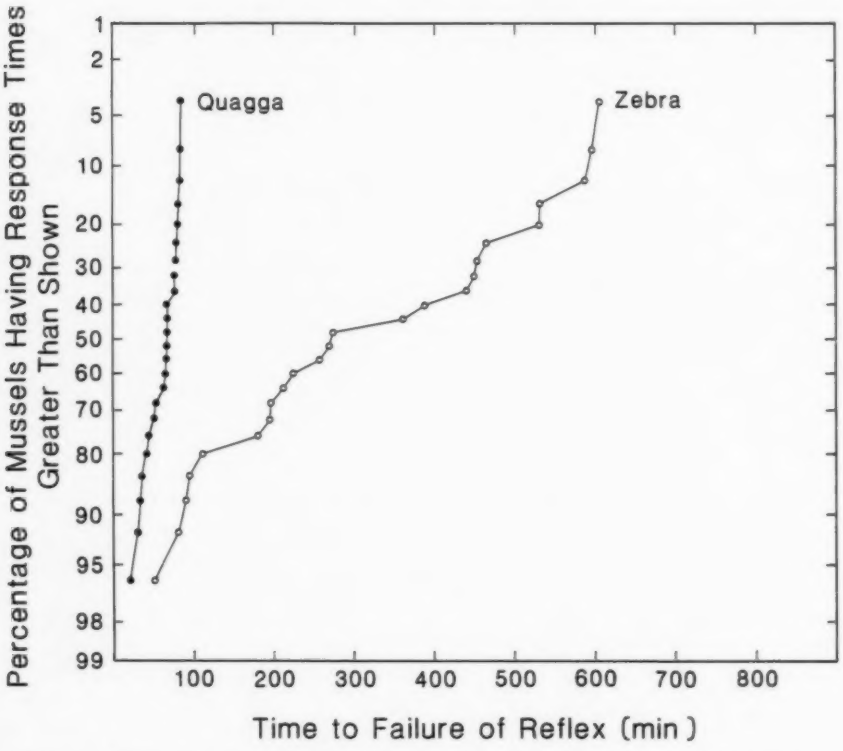



FIG. 2. Cumulative mortality of zebra and quagga mussels in a bath maintained at 32°C (shown as resistance times plotted on probability paper).

by taking the endpoint as the failure of a gaping mussel to close its valve when prodded with a glass probe (Basedow 1969; Fisher et al. 1991; Iwanyzki and McCauley 1993). It should be noted that a mussel does not gape when it is disturbed, but quickly closes its shell and remains closed. Moreover, this endpoint is not death but an event preceding it if heating were continued; test animals usually recover when rescued immediately (Griffiths 1991). Groups of three zebra mussels and three quagga mussels were tested simultaneously to ensure similar test conditions. The heating rate was 0.3°C/min, which is the rate commonly used to minimize thermal acclimation during the course of an experiment.

### Resistance Times at Constant Temperatures

In a second and separate method of determining thermal resistance, 25 of each dreissenid were transferred abruptly to the same upper (constant) lethal temperature bath and observed continuously. The times at which individuals failed to close their valves in response to the mechanical stimulation were recorded and plotted on probability paper. The individuals were removed

for further observation, measured for shell length, and entered into the collection of the Wilfrid Laurier Zoological Museum.

## Results and Discussion

### Behaviour of Mussels during Testing

At the beginning of each CTmax determination, the mussels lay on the bottom of the vessel with their valves closed; spontaneous attachment by byssal threads did not occur. With increasing temperature the valves opened and, when tapped, a mussel displayed the closing reflex with diminishing force up to the endpoint — the cessation of irritability. When returned to the acclimation bath, the closing reflex could once again be elicited after a short recovery time. We assume, therefore, that the cessation of irritability was not due to habituation, but rather to the effects of lethal water temperatures.

In the constant lethal temperature experiment, the mussels gaped within a short time of their introduction. During the course of the experiment the closing reflex became weaker until the valves no longer closed. After this endpoint had been reached, the mussels were returned to the acclimation vessel where they usually recovered.

### Comparison of CTmax

The mean CTmax of zebra mussels was 36.97 ± 0.63°C and that of quagga mussels was 36.42 ± 0.68°C. The results are presented for nine replicate runs. Three zebra mussels and three quagga mussels acclimated to 20°C were tested simultaneously (Table 1). In all but three instances (i.e., 3 of 27 comparisons), a higher temperature was required to elicit the cessation of irritability in zebra mussels as compared with quagga mussels. These differences were statistically significant at the $P < 0.001$ level (i.e. chi FD = 16.33 > chi FD[1,0.001] = 10.28). We conclude, therefore, that zebra mussels were more resistant thermally than quagga mussels, in terms of CTmax.

### Resistances Times of Mussels at a Constant Lethal Temperature

The average survival time of zebra mussels tested at 32°C was 275 min whereas that of quagga mussels was 75 min. These findings were consistent with the published upper lethal temperatures of zebra mussels (Iwanyzky and McCauley 1993). Two distinct curves resulted when the data were plotted on probability paper (Fig. 2), a procedure often used to describe the sequence of death of fish at lethal temperatures (Fry et al. 1946). This representation suggests that the response of quagga mussels was more homogeneous than those of zebra mussels. This was confirmed by the coefficient of variation (mean/standard deviation × 100%) of quagga mussels which was 28% whereas a value of 90% was obtained for zebra mussels. This is consistent with May and Marsden's (1992, p. 1505) assertion that "There is a significantly lower level of [electrophoretic] heterozygosity relative to zebra mussels and although the quagga mussel may have different environmental tolerances than the zebra mussels, they have a much narrower genetic base for different environmental response to the environment." A more comprehensive study would be necessary to explore the extent of this variability in response to upper lethal temperatures and to show the differences in thermal tolerances. The current work, however, establishes the distinctiveness of these dreissenids with respect to a physiologically important characteristic: resistance to upper lethal temperatures. Moreover, these results suggest that there may be important ecological differences associated with these two species of dreissenids, and perhaps they will become evident with the further colonization of North American ecosystems.

## Acknowledgements

We thank Dr. Bak Chauham of the Ontario Ministry of Energy for his encouragement. We also acknowledge the assistance of Bruno Polewski of Ontario Hydro's Nanticoke Thermal Generating Station. Phil Ryan and Jim Murphy of the Ontario Ministry of Natural Resources helped obtain specimens from Port Dover. This work was supported by funds from the Ontario Ministry of the Environment and Wilfrid Laurier University. Laurie MacRae-Mitchell prepared the drawings.

## References

Basedow, T. 1969. Über die Auswirkung von Temperaturschocks auf die Temperaturresistenz poikilothermer Wassertiere. Eine Untersuchung zum Problem der thermischen Schockanpassung bei Tieren. Int. Rev. Gesamten Hydrobiol. 54: 765–789.

Becker, C.D., and G. Genoway. 1979. Evaluation of the critical thermal maximum for determining the thermal tolerance of freshwater fish. Environ. Biol. Fishes 4: 245–256.

Beitinger, T.L., and R.W. McCauley. 1990. Whole animal physiological processes for the assessment of stress in fishes. J. Great Lake Res. 16: 542–575.

Cowles, R.B., and C.M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Nat. Hist. 83: 265–296.

Fisher, S.W., P. Stromberg, K.A. Bruner, and L.D. Boulet. 1991. Molluscidal activity of potassium to the zebra mussel *Dreissena polymorpha* toxicity and mode of action. Aquat. Toxicol. 20: 219–234.

Fry, F.E.J. 1956. The lethal temperature as a tool in taxonomy. Ann. Biol. 33 (5–6): 205–219.

Fry, F.E.J., J.S. Hart, and K.F. Walker. 1946. Lethal temperature relations for a sample of young speckled trout *Salvelinus fontinalis*. Univ. Toronto Stud. Biol. Ser. 54, Ont. Fish. Lab. Publ. 66: 1–62.

Griffiths, J.S. 1991. Control of zebra mussels by thermal shock. Rep. No. 92-65-K: 90 p. (Available from Ontario Hydro Research Division, Ontario Hydro)

Hart, J.S. 1952. Geographic variations of some physiological and morphological characters in certain freshwater fish. Univ. Toronto Stud. Biol. Ser. No. 60, Ont. Fish. Lab. Publ. 72: 1–79.

Hebert, P.D.N., B.W. Muncaster, and G.L. Mackie. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587–1591.

Hirshfield, C.R., C.R. Feldmeth, and D.L. Soltz. 1980. Genetic differences in physiological tolerances of Amargosa pupfish (*Cyprinodon nevadensis*) populations. Science (Wash., D.C.) 207: 999-1001.

Iwanyzki, S., and R.W. McCauley. 1993. Upper lethal temperatures of adult zebra mussels. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

Kott, E., R.W. McCauley, and G. Humphreys. 1980. Morphological and physiological (i.e. upper lethal temperatures) comparisons between *Notropis cornutus* (Mitchell) and *Notropis chrysocephalus* (Rafinesque) from the Grand River, Ontario. Can. J. Zool. 58: 2096–2101.

Ludyanskiy, M.L. 1993. Recent introductions of *Dreissena* into North America — The Caspian Sea/Black Sea connection, p. 699–704. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

May, B., and J.E. Marsden. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. 49: 1501–1506.

McCauley, R.W. 1958. Thermal relations of geographic races of *Salvelinus*. Can. J. Zool. 36: 655–662.

McCauley, R.W., and D.M. Kilgour. 1986. Reconciling the two methods of measuring upper lethal temperatures of fish. Environ. Biol. Fishes 16: 281–290.

Nalepa, T.F., and D.W. Schloesser [ed.] 1993. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

# Invasion of Lake Erie Offshore Sediments by *Dreissena*, and Its Ecological Implications

R. Dermott and M. Munawar

*Department of Fisheries and Oceans, Canada Centre for Inland Waters, Box 5050, Burlington, ON L7R 4A6, Canada*

Dermott, R., and M. Munawar. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Can. J. Fish. Aquat. Sci. 50: 2298–2304.

Large populations of the exotic rounded (noncarinate) shelled mussel of the genus *Dreissena* were found to exist on soft sediments collected throughout the central and eastern basins of Lake Erie during July and August 1992. Two different phenotypes were present on fine sediments (<150 μm) in the eastern basin. An elongated white morph was common on the profundal sediments beyond 40 m depth, while the "quagga" mussel was common on sand and sandy silt at depths between 10 and 30 m. Together with the carinated zebra mussel *Dreissena polymorpha*, which is very abundant on hard substrates in the sublittoral region, at least 80% of Lake Erie's bottom sediments have been invaded by *Dreissena*. Only that region of the central basin (near Cleveland) which undergoes periodic summer anoxia was not inhabited by this genus.

De grandes populations de moule exotique à coquille arrondie (non carénée) appartenant au genre *Dreissena* ont été observées sur des sédiments mous recueillis dans les bassins du centre et de l'est du lac Érié, en juillet et en août 1992. Deux phénotypes différents se trouvaient dans des sédiments fins (< 150 μm) dans le bassin de l'est. Une forme blanche allongée était courante sur les sédiments profonds à plus de 40 m de profondeur, tandis que la moule « quagga » était courant sur le sable et le limon sableux, à des profondeurs comprises entre 10 et 30 m. La moule zébrée carénée *Dreissena polymorpha* est très abondante sur les substrats durs de la région infralittorale; ensemble, les trois types de *Dreissena* ont colonisé au moins 80% des sédiments de fond du lac Érié. Seule la région du bassin central (près de Cleveland) qui subit des périodes d'anoxie en été n'était pas envahie par ce genre.

*Received February 8, 1993*
*Accepted June 23, 1993*
*(JB790)*

*Reçu le 8 février 1993*
*Accepté le 23 juin 1993*

The freshwater mussel *Dreissena polymorpha* has rapidly become widespread throughout the Laurentian Great Lakes since its initial discovery in Lake St. Clair during 1988 (Hebert et al. 1989). The species' spread has been assisted mostly by the downstream drift of its pelagic veliger larvae, as well as the passive transportation of adults when attached to drifting logs, vegetation, and especially boats. As a result, by 1991 the species was present at most major shipping ports throughout the Great Lakes. Mussels colonized the rocky shoals in the shallow western basin of Lake Erie (maximum depth 12 m) during 1987. By the fall of 1988, their distribution expanded along the north shore of the soft-bottomed central basin which has a depth of <25 m (Griffiths et al. 1991). The young of the next generation drifted into the deeper eastern basin (65 m) during the late summer of 1989 (Dermott et al. 1993).

*Dreissena polymorpha* preferably settle on hard substrates such as rock outcrops, stones, and submerged logs. They strongly attach by their byssal threads (Morton 1969a) which pull their flattened ventral surface tight against the substrate. In the absence of hard substrate, the mussels are able to survive as aggregations attached to pebbles, debris, and shells of unionid clams, or as clumps (druses) on other *Dreissena* (Morton 1969a; Bij de Vaate 1991; Mackie 1991). Populations are greatest in the littoral and sublittoral zones between 2 and 12 m (Stanczykowska 1964, 1977) with few individuals occurring below the thermocline (Mackie et al. 1989). Although *Dreissena* have been found to depths of 55 m, growth and reproduction did not occur at that depth in European lakes (Walz 1973, 1978). *Dreissena polymorpha*, as implied by its name, is highly variable in colour pattern and form (Morton 1969b) and can adapt to diverse environmental conditions (Smirnova 1991). In the Great Lakes, *D. polymorpha* has an exceptionally high level of genetic diversity in spite of an apparent single introduction (Hebert et al. 1989).

Recently, specimens of a morphologically and genetically distinct *Dreissena* were discovered in the Erie Canal and Lake Ontario by May and Marsden (1992), coexisting with but less numerous than the more widespread *D. polymorpha*. This second, yet unidentified species, given the working name "quagga" mussel by May and Marsden (1992), has a distinctive shell with convex ventral margin and lacks the carina between the ventral and lateral shell surfaces resulting in a rounded cross-section. *Dreissena polymorpha* has a flat or concave ventral margin and pronounced carina so that the ventral edge of the shell is perpendicular to the lateral, giving the zebra mussel a triangular cross-section. Furthermore, the quagga mussel has different ecological requirements than the zebra mussel (Domm et al. 1993).

During a 1992 multidisciplinary survey of Lake Erie (Munawar et al. 1992), the benthic samples revealed that *Dreissena* was very widespread throughout the lake, including soft offshore sediments. The purpose of this paper is to document this invasion, report the abundance of *Dreissena* in the centre of the lake, and describe a third possible taxon of *Dreissena* that is inhabiting the profundal zone of eastern Lake Erie. These deepwater mussels with distinctive shells were given the working name "profunda".

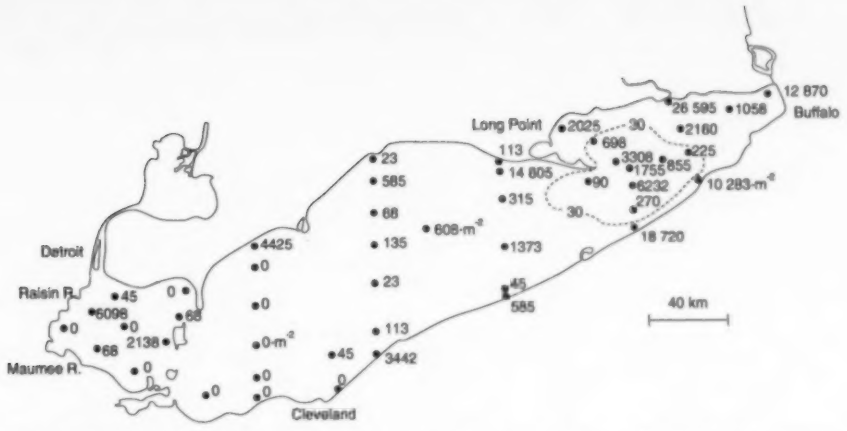



FIG. 1. Location of Lake Erie sample sites and abundance of *Dreissena* (no · $m^{-2}$) retained on 1-mm mesh, July–August 1992. The 30-m contour in the eastern basin is outlined.

## Methods

During July and August 1992, duplicate mini Ponar grabs (0.022 · $m^{-2}$) were collected from 47 sites in Lake Erie, including three sites in the deepest portion of the eastern basin (Fig. 1). The site locations were chosen by a research committee to give complete spatial coverage of the lake based on historical data sets of water chemistry, biological parameters, and physical variables (Vollenweider et al. 1974; Munawar and Burns 1976). All sites were situated in water deeper than 6 m. One benthic sample was collected at all the sites and two grabs were preserved at most sites with six sites having either both grabs combined together or only one grab collected. One site had three mini Ponar grabs collected and preserved. The samples were screened through a 0.15-mm net and the retained residue preserved in 8% formalin for later analysis.

Samples were later rescreened in the laboratory to tally the number and weight of the mussels retained on a 1-mm screen. The mussels were separated into zebra, quagga, and profunda based on the shape of shells. The size frequencies of the population inhabiting the profundal zone of Lake Erie were examined at two sites of 60 m depth. For these samples the lengths of both the mussels retained on the 1-mm mesh and the newly settled mussels retained on a 0.25-mm mesh were measured to the nearest 0.1 mm with a vernier caliper or ocular micrometer.

The following measurements were made using similar sized specimens of zebra, quagga, and profunda mussel collected from eastern Lake Erie in July 1992. Length was measured from the umbo to the posterior margin parallel to the long axis of the shell. Height as the maximum dorsal–ventral dimension was measured perpendicular to the length; width was the left–right dimension with both valves appressed. Dorsal length was the distance from the umbo to where the dorsal–posterior angle occurs or the dorsal margin curves beyond approximately 10°. Ventral length was from the umbo to junction of the ventral and posterior margins or a point where the ventral margin curved beyond 10°. Byssal position was the distance between the umbo and byssal groove.

## Results

### Distribution

The substrate retrieved at one site consisted of only *Dreissena*, probably inhabiting bedrock. Gravel was collected at four sites, medium sand (>200 μm in diameter) at five sites, and the remaining 37 sites were either fine sand (<200 μm), silt, clay, or fine profundal ooze. All of the sites sampled in the shallow western basin of Lake Erie were situated on fine silt or clay. In this survey, 36 of the 47 sites had *Dreissena* existing either as clumps of attached mussels or separate individuals. *Dreissena* were collected at all 15 sites in the eastern basin, 16 of 23 sites in the central basin, and five of the nine sites sampled in the western basin (Fig 1). The only area of Lake Erie inhospitable to *Dreissena* was the west end of the central basin near Cleveland (seven sites) and four sites in the western basin. Except for several sites in the profundal zone of the lake, chi-squared analysis of the replicated samples indicated that the mussel distribution was contagious, i.e., highly clumped ($\chi^2$ = 27–250). The maximum abundance of zebra mussel occurred on rocks and gravel along the shore of the eastern basin (Fig. 1).

The quagga mussel coexisted with the more abundant zebra mussel as a small percentage of the population in most of the central basin and on rocky and gravel substrates in the nearshore areas of the eastern basin. Quagga mussel were present along the north shore of Lake Erie as far west as Rondeau (Fig. 2), where they represented less than 0.3% of the *Dreissena* present; however, no specimens were found in western Lake Erie. Quagga mussel were most common near Long Point, where their populations ranged between 90 and 2200 · $m^{-2}$. In eastern Lake Erie, the proportion of quagga mussel was often over 50% of the *Dreissena* present, particularly on hard clay and sandy substrates at depths between 10 and 30 m (Table 1). In the eastern basin, neither zebra nor quagga mussel were found below 37 m.

### Profundal *Dreissena*

At all profundal sites in the eastern basin of Lake Erie (below 40 m depth), white mussel, also with rounded ventral edges,

TABLE 1. Depth distribution of *Dreissena* in samples from Lake Erie, 1992. Only animals retained on 1-mm mesh counted. Standard errors in parentheses.

| Depth (m) | No. of grabs | Total *Dreissena* ($m^{-2}$) | Percent with rounded ventrals | Wet biomass including shell ($g \cdot m^{-2}$) |
|---|---|---|---|---|
| 0–5 | 0 | No samples | | |
| 5–10 | 18 | 3096.3 (1342.7) | 10.6 (5.5) | 220.85 (11.27) |
| 10–20 | 31 | 3443.4 (1644.3) | 12.4 (7.5) | 340.90 (123.79) |
| 20–30 | 24 | 536.4 (149.8) | 36.5 (9.7) | 42.89 (13.43) |
| 30–40 | 6 | 397.3 (146.2) | 84.8 (11.7) | 21.90 (8.35) |
| 40–63 | 10 | 2448.0 (748.5) | 100.0 (0.0) | 107.45 (41.25) |

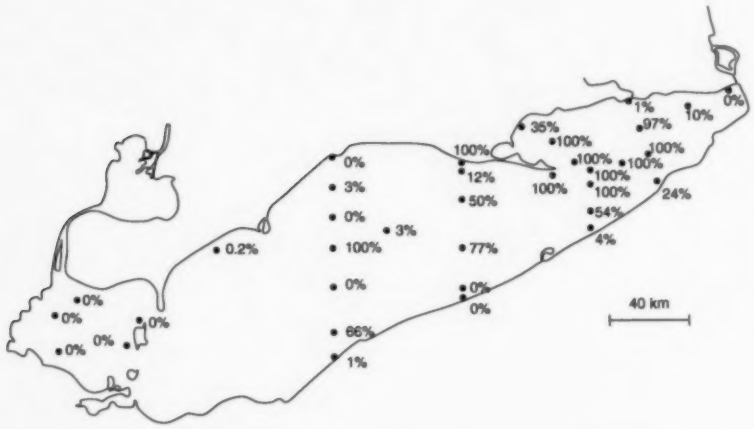



FIG. 2. Percentage of *Dreissena* with rounded ventral margins at the sites with live *Dreissena*, July 1992. This includes both the quagga and profunda mussel.

represented 100% of the *Dreissena* present. In the profundal zone, populations of this morph averaged $2448 \pm 1497 \cdot m^{-2}$ (Table 1). Like the quagga mussel described by May and Marsden (1992), these profunda mussel have a rounded ventral margin, the shells lacking the carina of the more common zebra mussel (Fig. 3). However, the ventral margin of the shell on the profunda specimens is almost straight or slightly convex, as compared with the often strongly convex ventral margin on the quagga mussel. Viewed from the ventral, the ventral margin between the shells is straight and not sinusoidal as on the quagga mussel (Domm et al. 1993). In addition, an obvious anterio-ventral swelling of the shell exists between the umbo and pedal gape on the profunda specimens (Fig. 4). The off-centre beak and swollen ventral are obvious on specimens as small as 1 mm.

The shells of the *Dreissena* inhabiting the soft profundal sediments have little pigment, or pigment limited to a few longitudinal dark bands along the dorsal side of the shell. A slight obtuse, rounded ridge extends to the dorsal side of the shell. The thin shell is elongate with an elliptically curved posterior margin. The umbo is terminal, acute, and the anterior shell is modioliform, swollen anterioventrally between the pedal gape and umbo. The ventral carina is lacking, and the ventral margin is almost straight or slightly convex, never concave behind the umbo but straight to approximately 80% of the length. The right valve is slightly larger than the left posteriorly of the pedal gape. The dorsal margin is straight posteriorly to beyond 50% of the length, forming an obtuse angle, and then curves elliptically along the posterior margin. The septum is broad with no apophysis, and the hinge plate is without teeth. The pallial line has a slight truncated sinus. The exhalant siphon is short, the larger inhalant siphon extendible up to 40% of the shell length. The byssal groove and pedal gape are both displaced anteriorly toward the umbo, at about 26% of the shell length.

Zebra mussel had the widest shells (left–right) of the three dreissenids (Table 2). Quagga mussel shells were significantly higher (dorsal–ventral) than the other two mussels. The profunda mussel had significantly narrower shells and shorter byssal–umbo distance (range 1.8–4.1 mm), as well as significantly smaller ratios of width to length (W/*L*, range 0.29–0.35) and byssal position to length (*B*/*L*, range 0.14–0.34).

## Mode of Life and Size Distribution

Where zebra mussel or the rounded quagga mussel occur on soft sand or silt, the majority of animals occur as druses or clumps held together by their byssal threads. This was the case at all the sites in Lake Erie above 30 m. The profunda *Dreissena* occurred freely in the samples, rarely connected to each other by byssal threads. At several profundal sites, bands of iron and manganese oxide deposits occurred near the umbones of the larger shells. Similar oxide deposits often occur on the shells of *Pisidium* (Herrington 1962), outlining the redox zones in the sediment at the level where the clams burrow. These oxide bands near the umbones of the profunda mussel indicate that the shells are

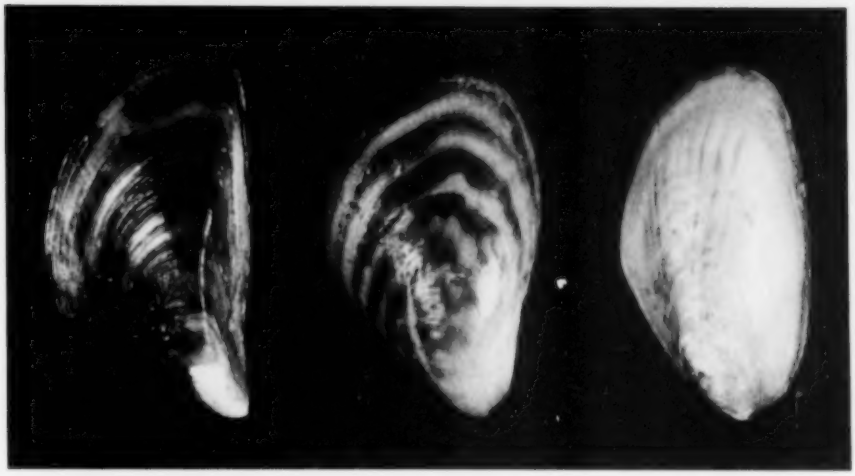


FIG. 3. Photograph of (left to right) a zebra mussel (*D. polymorpha* s.s) and the two morphs with rounded ventral margins, May and Marsden's quagga and morph and the profunda morph from eastern Lake Erie. All specimens are of the same scale: the profunda specimen is 13.5 mm long.

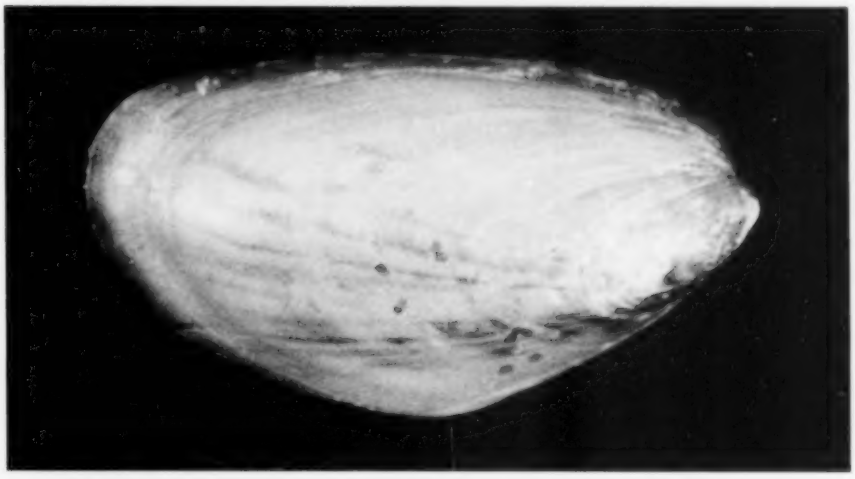


FIG. 4. Specimen of profunda mussel from 48 m in eastern Lake Erie; shell length is 13.5 mm.

oriented siphons upward, with the umbones buried at least 3–6 mm into the sediments. Subsequent collections revealed that the anteriorly displaced foot allows animals to move through soft sediment with their shells half buried and siphons oriented vertically.

Profunda mussel were between 0.5 and 16 mm long. Although these profundal mussel were not large compared with either zebra or quagga mussel occurring nearshore (up to 30 mm), there were two distinct size classes present during summer 1992 (Fig. 5). At 60 m depth, animals longer than 10 mm were at least 1 yr old, possibly 2 if they settled during the late summer of 1990. No animals would be older than 3 yr, as *Dreissena* first entered the east basin of Lake Erie in 1989. During midsummer, newly settled mussels (0.3–2 mm) were present in the profundal samples, sometimes attached to the larger mussels indicating recent reproduction. Densities of mussels <2 mm in length averaged 18 348 · $m^{-2}$ (range 3 105–40 410) at four profundal sites examined in detail.

## Discussion

The morphometric description of the *Dreissena* from the profundal zone of Lake Erie closely matches that of *Dreissena distincta* Andrusov (Zhadin 1952) which inhabits the Caspian Sea down to 200 m. *Dreissena distincta* is provisionally synonymous to the *Dreissena (Pontodreissena) rostriformis* Deshayes (Nuttall 1990); however, the profunda mussel in Lake Erie do not have the rostrate process of *D. rostiformis* (Archambault-Guezou 1976, plate II; Nuttall 1990, plate I). In eastern Lake Erie, between 28 and 37 m, the shape of the profundal mussel intergrades with that of the quagga mussel; the profunda mussel may be a phenotype adapted to survival on soft sediments. Genetic

TABLE 2. Morphometrics (averages) of dreissenids from eastern Lake Erie, 1992. Significant differences between pairs of mussels (Z, zebra; Q, quagga, P, profunda) indicated by $*p < 0.01$ and $**p < 0.001$ according to *t*-tests. Standard errors in parentheses.

| | Zebra | Z vs. Q | Quagga | Q vs. P | Profunda | Z vs. P |
|---|---|---|---|---|---|---|
| *n* | 15 | | 15 | | 15 | |
| Length (*L*)(mm) | 12.99 (0.483) | | 13.47 (0.494) | | 12.69 (0.297) | |
| Height (*H*)(mm) max. | 6.88 (0.299) | * | 8.29 (0.328) | * | 6.88 (0.183) | |
| Width (W)(mm) max. | 6.37 (0.304) | | 5.28 (0.236) | * | 4.19 (0.127) | * |
| Dorsal length (*D*) (mm) | 7.09 (0.296) | | 8.41 (0.356) | | 7.46 (0.281) | |
| Ventral length (V) (mm) | 11.98 (0.493) | * | 10.18 (0.334) | | 10.62 (0.287) | |
| Byssal position (*B*) (mm) | 4.90 (0.303) | | 4.74 (0.222) | ** | 3.26 (0.178) | * |
| Ratios | | | | | | |
| *H*/*L* | 0.53 (0.010) | | 0.61 (0.008) | ** | 0.54 (0.008) | |
| W/*L* | 0.49 (0.009) | ** | 0.39 (0.010) | ** | 0.33 (0.004) | * |
| W/*H* | 0.92 (0.012) | ** | 0.64 (0.019) | | 0.61 (0.007) | * |
| *D*/*L* | 0.55 (0.014) | ** | 0.62 (0.011) | | 0.58 (0.009) | |
| V/*L* | 0.92 (0.014) | ** | 0.76 (0.024) | | 0.84 (0.015) | * |
| *B*/*L* | 0.37 (0.015) | | 0.35 (0.010) | ** | 0.26 (0.015) | * |

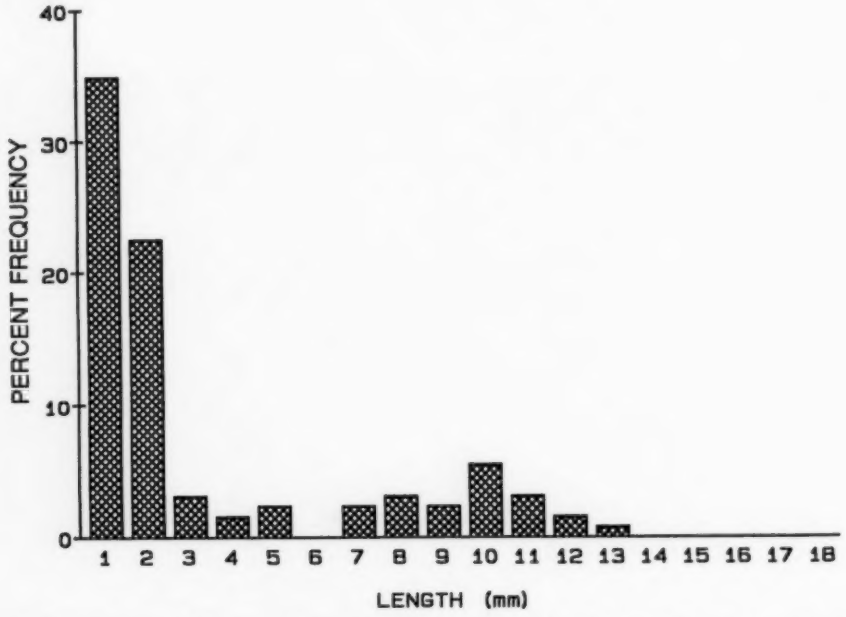



FIG. 5. Size frequency of profunda *Dreissena* inhabiting eastern Lake Erie at 60 m depth in July 1992. In addition to the mussels retained on a 1-mm mesh, newly settled mussels retained on a 0.25-mm mesh were included in the size frequency analysis: $n = 129$.

and physiological studies have been undertaken on the deep-water mussels in Lake Erie to determine the taxonomical relationships,[1] and the living Palaearctic *Dreissena* should be reviewed to resolve the identify of both the quagga and profunda mussel (Ludyanskiy 1991; May and Marsden 1992).

The hypolimnion of the west end of the central basin experiences periodic summer anoxia (Burns 1976; Charlton 1987) which severely restricts the benthic community present to mostly tubificid worms. Similar conditions may also be the limiting factor at the three sites in the western basin without live *Dreissena*. Two of these sites were below 10 m depth, one of which (midbasin site) had over 100 dead but still closed *D. polymorpha* shells, perhaps killed by periodic anoxia. The third site was close to the Maumee and Raisin River mouths near Toledo (Ohio) which historically had a highly eutrophic benthic community (Carr and Hiltunen 1965). Their abundance on the soft bottom of Lake Erie indicates that *D. polymorpha* is capable of colonizing soft bottom where sedimentation rate and oxygen conditions are not restrictive. Similarly, *D. polymorpha* have been found as druses on muddy sand and silt in shallow Lake St. Clair (Hunter and Bailey 1992) and the western basin of Lake Erie (Nalepa and Schloesser 1993). Hunter and Bailey (1992)

[1]Recent genetic studies could not differentiate the Lake Erie profunda morph from the quagga mussel (A. Spindle, Cornell Laboratory for Ecological and Evolutionary Genetics, Ithaca, N.Y., personal communications).

found that zebra mussel colonized soft substrates in shallow Lake St. Clair by lateral extension of the colonies (druses) from an original small piece of hard substrate, usually a unionid shell. *Dreissena* populations in Lake St. Clair had attained densities of 20 000 · $m^{-2}$ by 1991, resulting in an increase in water clarity, increased macrophyte growth, and changing benthic fauna reflecting more oligotrophic conditions (Griffiths 1993).

A benthic survey of Lake Ontario (Dermott 1993) revealed that by November 1990, both zebra and quagga mussel were present at a few sites along the south shore eastward from the Niagara River. Ponar samples collected from a depth of 107 m (off Oswego) contained both zebra mussel and the rounded quagga mussel (Dermott 1993); at the time, these 4-mm-long mussels were presumed to have drifted to the site on debris, rather than settling and growing at that depth. However, quagga mussel have since colonized the south side of Lake Ontario (May and Marsden 1992; Mills et al. 1993) surviving at depths down to 130 m.

Walz (1978) found that the mussels inhabiting the steep slopes of Lake Constance at 50 m depth, unlike the *Dreissena* in the profundal zone of Lake Erie, had an insufficient food source for either growth or reproduction at that depth. The survival and growth of the newly settled profunda mussel in Lake Erie will result in populations of several thousand animals per square metre depending on the intraspecific competition for settling phytoplankton. During 1992, nearshore settling of *Dreissena* did not occur until early October (Ontario Hydro and DFO, unpublished data), while newly settled young were present in the profundal zone in August. This suggests that *Dreissena* inhabiting the deeper water of Lake Erie are reproductively separated from those nearshore due to both seasonal cues and the thermocline. As profunda mussel, and to a lesser degree quagga mussel, are able to grow in cooler water, their larvae may take longer to develop and hence remain pelagic longer than zebra mussel larvae released in warmer water.

The invasion of large populations of *Dreissena* on the soft sediments in deep water has several major implications. The ability to grow in water below 10°C (Morton 1969b) and reproduce at temperatures below 13°C (Strayer 1991) will expand the potential range of *Dreissena* northward to colder regimes, perhaps as far north as Great Slave Lake. The lower water temperatures will not discourage population growth in the upper Great Lakes. The survival, growth, and apparent reproduction of *Dreissena* at much greater depths than reported earlier will potentially allow the genus to colonize most substrates in the Great Lakes above 60 m. The soft sediments in the shallower south ends of Lakes Michigan and Huron and the east end of Lake Ontario may support mussel populations of 500–1000 animals · $m^{-2}$. Their depth tolerance also means that deepwater intakes (below 20 m) are no longer safe from mussel infestation. Although restricted by location, this control option is considered an economic alternative to expensive mechanical cleaning and chemical control of mussel infestation at water intakes (Yount 1991), a financial burden that can cost public water facilities on Lake Erie $150,000 (U.S.) per year (LePage 1993).

The *Dreissena* in the Great Lakes show ecological separation by temperature preference (Domm et al. 1993) and the ability to survive on different substrates. The high, flat shells of quagga mussel may be an adaption to allow epibenthic suspension feeding on sandy substrates, where the species is common in both Lakes Erie and Ontario. The more elongated, modioliform shells of profunda mussel allow colonization of soft mud paralleling the endobyssate mytilid *Modiolus* with similar morphological adaptions in shell shape for partial burial in soft substrate (Stanley 1972), as well as the form of several extinct Pontian Dreissenoidea (Morton 1993; L. Kalas, in preparation). These habitats are not favourable to the zebra mussel which have colonized all the hard rocky substrates nearshore. However, the ability of the various *Dreissena* to survive on the soft sediments of Lake Erie results in at least 80% of the total bottom area of Lake Erie now being inhabited by the genus. The area below 20 m, formally considered inhospitable to *Dreissena*, represents 48% of Lake Erie's total area (Robertson and Jordan 1978). As a result, the filtering activities of the dreissenids now impact both the ecology of the littoral shoals and the profundal zone of the lake, greatly increasing the total amount of Lake Erie phytoplankton consumed by this one genus. This shift will affect both the benthic and zooplankton food web dynamics of Lake Erie.

The colonization of the profundal zone of Lake Erie has changed the benthic community at sites beyond 40 m depth. By 1992, profunda mussel represented between 20 and 90% of the benthic biomass (dry shell-free) at the four sites examined in detail (Dermott 1993). The competition for space and food limits the traditional profundal fauna, in particular the abundance of the burrowing amphipod *Diporeia hoyi* (formally *Pontoporeia*), which was historically dominant in the eastern basin (Barton 1988; Dermott 1993). This alteration in the composition of the offshore benthos due to direct competition for settling phytoplankton will impact the deepwater fisheries of eastern Lake Erie, which is the largest natural freshwater fishery in North America (FAO 1989). Therefore, it is imperative to monitor the changes in the offshore habitat of the Great Lakes rather than only the inshore regions so that appropriate management strategies can be devised.

## Acknowledgements

We thank Dr. Len Kalas for advice on the taxonomy and morphology of *Dreissena* and suggesting the name "profunda" for the deepwater mussels. We thank the crews of the Canadian survey ships *Limnos* and *Advent* for collection of the samples. The photographs are by Alan Wormington. Mr. H.F. Nicholson assisted with the technical editing of the manuscript. We thank the anonymous referees for providing valuable criticism.

## References

ARCHAMBAULT-GUEZOU, J. 1976. Présence de Dreissenidae euxiniques dans les dépôts à Congéries de la vallée du Rhône et sur le pourtour du bassin méditerranéen. Implications biogéographiques. Bull Soc. Géol. France 18: 1267–1276.

BARTON, D.R. 1988. Benthic fauna from Great Lakes Institute cruises on Lake Erie — 1963, 1964, 1965. Can. Tech. Rep. Fish. Aquat. Sci. 1635: v + 61 p.

BIJ DE VAATE, A. 1991. Distribution and aspects of population dynamics of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in the Lake IJsselmeer area (The Netherlands). Oecologia 86: 40–50.

BURNS, N.M. 1976. Temperature, oxygen and nutrient distribution patterns in Lake Erie, 1970. J. Fish. Res. Board Can. 33: 485–511.

CARR, J.F., and J.K. HILTUNEN. 1965. Changes in the bottom fauna of western Lake Erie from 1930 to 1961. Limnol. Oceanogr. 10: 551–569.

CHARLTON, M.N. 1987. Lake Erie oxygen revisited. J. Great Lakes Res. 13: 697–708.

DERMOTT, R. 1993. Distribution and ecological impact of "quagga" mussels in the lower Great Lakes. Proceedings of Third International Zebra Mussel Conference. Electrical Power Research Institute Rep. TR-102077.

DERMOTT, R., J. MITCHELL, I. MURRAY, AND E. FEAR. 1993. Biomass and production of zebra mussels (*Dreissena polymorpha*) in shallow waters of northeastern Lake Erie, p. 399–413. *In* T.F. Nalepa and D.W. Schloesser

[ed.]. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

DOMM, S., R.W. MCCAULEY, E. KOTT, AND J.D. ACKERMAN. 1993. Physiological and taxonomic separation of two dreissenid mussels in the Laurentian Great Lakes. Can. J. Fish Aquat. Sci. 50: 2294–2297.

FAO. 1989. Fisheries statistics, catches and landings. FAO Fish. Ser. 68: 517 p.

GRIFFITHS, R.W. 1993. Effects of zebra mussels (*Dreissena polymorpha*) on benthic fauna of Lake St. Clair, p. 415–437. *In* T.F. Nalepa and D.W. Schloesser [ed]. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

GRIFFITHS, R.W., D.W. SCHLOESSER, J.H. LEACH, AND W.P. KOVALAK. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes Region. Can. J. Fish. Aquat. Sci. 48: 1381–1388.

HEBERT, P.D.N., B.W. MUNCASTER, AND G.L. MACKIE. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. 46: 1587–1591.

HERRINGTON, H.B. 1962. A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). Misc. Publ. Mus. Zool. Univ. Mich. 118: 1–74.

HUNTER, R.D., AND J.F. BAILEY. 1992. *Dreissena polymorpha* (zebra mussels): colonization of soft substrata and some effects on unionid bivalves. Nautilus 106: 60–67.

LEPAGE, W.L. 1993. The impact of *Dreissena polymorpha* on waterworks operations at Monroe, Michigan: a case history, p. 333–358 *In* T.F. Nalepa and D.W. Schloesser [ed]. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

LUDYANSKIY, M.L. 1991. Are *Dreissena polymorpha* and *Dreissena bugensis* synonymous? *Dreissena polymorpha*. Inf. Rev. N.Y. Sea Grant 2(6).

MACKIE, G.L. 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha*, in relation to native bivalves and its potential impact in Lake St. Clair. Hydrobiologia 219: 251–268.

MACKIE, G.L., W.N. GIBBONS, B.W. MUNCASTER, AND I.M. GRAY. 1989. The zebra mussel, *Dreissena polymorpha*: a synthesis of European experiences and a preview for North America. Water Resources Branch, Great Lakes Section, Ontario Ministry of the Environment, Toronto, Ont. 76 p + appendices.

MAY, B., AND J.E. MARSDEN. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. 49: 1501–1506.

MILLS, E.L., R.M. DERMOTT, E.F. ROSEMAN, D. DUSTIN, E. MELLINA, D.B. CONN, AND A.P. SPIDLE. 1993. Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. Can. J. Fish. Aquat. Sci. 50: 2305–2314.

MORTON, B.S. 1969a. Studies on the biology of *Dreissena polymorpha* Pall. I. General anatomy and morphology. Proc. Malacol. Soc. Lond. 38: 301–320.

MORTON, B.S. 1969b. Studies on the biology of *Dreissena polymorpha* Pall. III. Population dynamics. Proc. Malacol. Soc. Lond. 38: 471–481.

MORTON, B. 1993. The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenoidea, p. 185–215. *In* T.F. Nalepa and D.W. Schloesser [ed]. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

MUNAWAR, M., S. BRANDT, G. SPRULES, AND D. STEWART. 1992. Lake Ontario trophic transfer (LOTT) study: an emergence of an ecosystemic and multi-disciplinary research strategy in the Great Lakes. Abstract of presentation at 35th Conference of International Association for Great Lakes Research, May 3, 1992, Waterloo, Ont.

MUNAWAR, M., AND N.M. BURNS. 1976. Relationships of phytoplankton biomass with soluble nutrients, primary production, and chlorophyll *a* in Lake Erie, 1970. J. Fish. Res. Board Can. 33: 601–611.

NALEPA, T.F., AND D.W. SCHLOESSER [ED.] 1993. Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p. Colour plates 11 and 28.

NUTTALL, C.P. 1990. Review of the Caenozoic heterodont bivalve superfamily Dreissenaceae. Palaeontology 33: 707–737.

ROBERTSON, D.G., AND D.E. JORDAN. 1978. Digital bathymetry of Lakes Ontario, Erie, Huron, Superior and Georgian Bay. Environ. Can. Can. Centre Inland Waters Rep. 28 p.

SMIRNOVA, N. 1991. Biology and ecology of *Dreissena polymorpha* from the European USSR. *In* J.D. Yount [ed]. Ecology and management of the zebra mussel and other introduced aquatic nuisance species. U.S. Environ. Protect. Agency EPA/600/3-91/003: 8 p.

STANCZYKOWSKA, A. 1964. On the relationship between abundance, aggregations and conditions of *Dreissena polymorpha* Pall. in 36 Mazurian lakes. Ekol. Pol. 12: 653–690.

STANCZYKOWSKA, A. 1977. Ecology of *Dreissena polymorpha* (Pallas) (Bivalvia) in lakes. Pol. Arch. Hydrobiol. 24: 461–530.

STANLEY, S.M. 1972. Functional morphology and evolution of byssally attached bivalve mollusks. J. Paleontol. 46: 165–212.

STRAYER, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish. Aquat. Sci. 48: 1389–1395.

VOLLENWEIDER, R.A., M. MUNAWAR, AND P. STADELMANN. 1974. A comparative review of phytoplankton and primary production in the Laurentian Great Lakes. J. Fish. Res. Board Can. 31: 739–762.

WALZ, N. 1973. Studies on the biology of *Dreissena polymorpha* Pallas in the Lake of Constance. Arch. Hydrobiol. 42(Suppl.): 452–482.

WALZ, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. II. Reproduction. Arch. Hydrobiol. 55(Suppl.): 106–119.

YOUNT, J.D. 1991. Ecology and management of the zebra mussel and other introduced aquatic nuisance species. Report of EPA Workshop, Sept. 26–28, 1990, Saginaw, Michigan. U.S. Environ. Protect. Agency EPA/600/3-91/003.

ZHADIN, V.I. 1952. Mollusks of fresh and brackish waters of the U.S.S.R. keys to the fauna of the U.S.S.R. Zool. Inst. Izd. Akad. Nauk S.S.S.R. Moskva-Leningrad 46: 376 p.

# Colonization, Ecology, and Population Structure of the "Quagga" Mussel (Bivalvia: Dreissenidae) in the Lower Great Lakes

Edward L. Mills

*Department of Natural Resources, Cornell Biological Field Station, 900 Shackelton Point Road, Bridgeport, NY 13030, USA*

Ron M. Dermott

*Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries, P.O. Box 5050, Burlington, ON L7R 4A8, Canada*

Edward F. Roseman[1] and Donna Dustin[2]

*Department of Natural Resources, Cornell Biological Field Station, 900 Shackelton Point Road, Bridgeport, NY 13030, USA*

Eric Mellina

*McGill University, Department of Biology, 1205 Dr. Penfield, Montreal, QB H3A 1B1, Canada*

David Bruce Conn

*Department of Biology, The University of the South, Sewanee, TN 37375, USA*

and Adrian P. Spidle

*Department of Natural Resources, Cornell Biological Field Station, 900 Shackelton Point Road, Bridgeport, NY 13030, USA*

Mills, E.L., R.M. Dermott, E.F. Roseman, D. Dustin, E. Mellina, D.B. Conn, and A.P. Spidle. 1993. Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. Can. J. Fish. Aquat. Sci. 50: 2305–2314.

An invasive dreissenid mussel given the working name of "quagga" has a present (spring 1993) distribution in the Laurentian Great Lakes from the western basin of Lake Erie to Quebec City. In Lake Erie, quaggas were collected as early as 1989 and now are most common in the eastern basin. In Lakes Erie and Ontario, proportions of quaggas increased with depth and decreasing water temperature. In the eastern basin of Lake Erie, quaggas outnumbered zebra mussel (*Dreissena polymorpha*) by 14 to 1 in deeper waters (>20 m). In Lake Ontario, quaggas were observed at depths as great as 130 m, and both quagga and zebra mussel were found to survive at depths (>50 m) where temperatures rarely exceed 5°C. Quaggas were sparse or absent along inland waterways and lakes of New York State. Mean shell size of quagga mussel was larger than that of zebra mussel at sites in the Niagara River, Lake Ontario, and the St. Lawrence River. The largest quaggas (38 mm) were observed in the St. Lawrence River at Cape Vincent.

Une moule envahissante du groupe des dreissénidés, appelée provisoirement « quagga » occupe à l'heure actuelle (printemps 1993) les Grands Lacs depuis le bassin de l'ouest du lac Érié jusqu'à la ville de Québec. Dans le lac Érié, des quaggas ont été prélevées dès 1989 et elles sont maintenant très courantes dans le bassin de l'est. Dans les lacs Érié et Ontario, la proportion de quagga a augmenté avec la profondeur et la baisse de la température. Dans le bassin de l'est du lac Érié, les quaggas sont plus nombreuses que les moules zébrées (*Dreissena polymorpha*), la proportion s'établissant à 14 pour 1 en eau profonde (> 20 m). Dans le lac Ontario, des quaggas ont été observées à des profondeurs atteignant 130 m; les quaggas et les moules zébrées peuvent survivre à des profondeurs (> 50 m) où la température dépasse rarement 5°C. Les quaggas étaient rares ou absentes le long des voies navigables de l'intérieur et dans les lacs de l'État de New York. La taille moyenne de la coquille des quaggas était supérieure à celle des moules zébrées sur certains sites du Niagara, du lac Ontario, et du fleuve Saint Laurent. Les quaggas les plus grosses (38 mm) ont été observées dans le fleuve Saint-Laurent au niveau de cap Vincent.

*Received April 15, 1993*
*Accepted June 29, 1993*
*(JB887)*

*Reçu le 15 avril 1993*
*Accepté le 29 juin 1993*

Scientists have been concerned for decades about the risks associated with introduced organisms. In the Great Lakes, a total of 139 nonindigenous aquatic organisms have been identified as established since the early 1800s (Mills et al. 1993). Of this total, almost one third have been introduced in the past 30 yr, a surge coinciding with the opening of the St. Lawrence Seaway in 1959. Introduced mollusks have had both ecological and economic impacts on the Great Lakes ecosystem (Griffiths

[1]Present address: Michigan State University, Department of Fisheries and Wildlife, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA.

[2]Present address: Department of Biology, University of Saskatoon, Saskatoon, SK S7N 0W0, Canada

FIG. 1. Sampling locations of quagga (Q) and zebra (Z) mussel in the lower Great Lakes and inland waterways of New York State during 1992. ● = quagga and zebra mussel absent; ○ = quagga and/or zebra mussel present (asterisk represents the first siting of the quagga mussel).

et al. 1991). Most notable examples include *Bithynia tentaculata*, a biofouling organism known as the faucet snail which abounded in the late 1800s (Baker 1902, 1928), and the zebra mussel (*Dreissena polymorpha*) which became established in the Great Lakes in 1986 (Griffiths et al. 1991).

In 1991, a second species of *Dreissena* was discovered in Lake Ontario and adjacent waterways (May and Marsden 1992). The second species was given the working name of "quagga" after an extinct African relative of the zebra. Morphologically, the most distinguishing feature of the quagga is that it lacks the flattened ventral side characteristic to the zebra mussel. There also appears to be a physiological separation between the two mussels, as Domm et al. (1993) have shown that upper lethal temperatures of zebra mussel were significantly higher than those of quagga mussel. To date, neither North American nor European taxonomists have made a positive identification of the quagga mussel, due in part to problems associated with dreissenid taxonomy (e.g., Zhadin 1952; Marelli and Gray 1985; Nuttall 1990; Marelli 1991; Ludyanskiy 1991, 1993). Recently, however, electrophoretic analyses have matched mussel tissues from southern reaches of the former Soviet Union with specimens of North American quagga (B. May, Cornell Laboratory for Ecological and Evolutionary Genetics, Cornell University, Ithaca, N.Y., personal communication), and this important finding may lead to the discovery of further differences associated with the two dreissenid species.

Currently, the demography of the quagga mussel is unknown. The objective of this study was to provide information on the colonization, ecology, and population structure of the quagga mussel in its known range in North America and to compare the distribution, habitat characteristics, and population size structure of quagga and zebra mussel.

## Methods

A total of 53 sites were sampled for quagga and zebra mussel from May to November 1992 (Fig. 1). Sites were located from Lake St. Clair to Quebec City in the Laurentian drainage and through the Erie–Barge Canal, Finger Lakes, and the Mohawk River to the Hudson River drainage of New York State. Specimens of both species from the May 1992 collections were confirmed by electrophoretic identification. In 1991 and 1992, samples of mussels were obtained from navigation buoys in Lakes Erie and Ontario, when the buoys were removed from the water for the winter (December until March). In addition, *Dreissena* samples were collected in Lake Erie near Port Colborne between September 1989 and October 1992, the mouth of the Niagara River in July 1990, along the south side of Lake Ontario in October 1990, and from navigation buoys in Lakes Erie and Ontario in 1990.

Methods of mussel collection varied among the 53 sites sampled from May to September 1992 (Table 1). In most areas inhabited by quagga and zebra mussel, a random mixture of a few hundred to several thousand live mussels were obtained for analysis from each site. In Lake Erie, mussels were collected from two different depths (8 and 27 m) whereas in Lake Ontario, 10 depths (8–130 m) were sampled. Bottom water temperature was measured at each site using either a YSI telethermometer or an electro-bathythermograph. Mussels were frozen and later sorted by species, counted, and total length measured to the nearest millimetre along the ventral margin.

The proportion of quagga and zebra mussel collected at individual sites in Lake Ontario was compared between spring and fall 1992 using chi-square. Differences in mean shell size between species was tested using a *t*-test whenever the test assumptions of normality and homoscedasticity were not violated. In cases where the length–frequency distributions departed

TABLE 1. Locations, number of sites, and methods used to collect quagga and zebra mussel, May–November 1992.

| Location | Number of sites | Method of collection |
|---|---|---|
| Lake St. Clair | 4 | Hand pick |
| Detroit River | 2 | Navigation buoys, hand pick |
| Lake Erie | | |
| Van Buren | 1 | Bottom trawl[a] |
| Brockton Shoal | 1 | Bottom trawl[a] |
| Other sites | 10 | Navigation buoys, hand pick |
| Niagara River | | |
| Upstream of Niagara Falls | 1 | Hand pick |
| Youngstown | 1 | Eckman grab, hand pick |
| Lake Ontario (Olcott, Thirty Mile Point, Rochester, Smoky Point, Fairhaven, Nine Mile Point, and Mexico Bay) | 7 | Bottom trawl[b] |
| Lake Ontario (Cape Vincent) | 1 | Benthic sled[c] |
| Lake Ontario (other sites) | 2 | Navigation buoys, hand pick |
| St. Lawrence River (all sites) | 5 | SCUBA diver, hand pick |
| Hudson River (all sites) | 2 | SCUBA diver, hand pick |
| Oneida Lake | 1 | SCUBA diver, hand pick |
| Cross Lake | 1 | SCUBA diver, hand pick |
| Erie–Barge Canal (all sites) | 4 | Hand pick |
| Onondaga Lake | 1 | Hand pick |
| Cayuga–Seneca Canal | 1 | SCUBA diver, hand pick |
| Finger Lakes (Canandaigua, Cayuga Conesus, Honeoye, Keuka, Owasco, Seneca, and Skaneateles) | 8 | SCUBA diver, hand pick |
| Total number of sites | 53 | |

[a]10-m head rope, cod end is 9-mm stretch mesh (Culligan et al. 1992).
[b]12-m head rope, cod end is 9-mm stretch mesh (O'Gorman et al. 1991)
[c]Hayes (1983).

from normality, a *G*-test was used to compare distributions (Sokal and Rohlf 1981). The Bonferroni correction was used in order to maintain the overall error rate at 0.05 (Snedecor and Cochran 1980). The relationship between lake depth, temperature, and proportion of quagga mussel for these samples was examined using regression analysis.

## Results

### First Sitings of Quagga Mussel

The first suspected occurrence of quagga mussel was in September 1989, when one quagga (length 3.8 mm) was found at the littoral site near Port Colborne, Lake Erie (Fig. 1). At this site, the proportion of quagga mussel among the settled *Dreissena* gradually increased from 2.3% during 1990 to 6.6% by October 1992 (Fig. 2). In 1990, quaggas were also present (3.5%) among mussels removed from a buoy at Port Stanley, Lake Erie, and two quaggas (lengths 8.1 and 12.2 mm) were collected at Niagara-on-the-Lake during July. During the fall of 1990, buoy and Ponar samples collected in Lake Ontario indicated that *Dreissena* were only present along the south shore between the Welland Canal and the east end of the lake (Fig. 3). Quagga mussel were present at many of these sites, in proportions ranging from 1.1% at the Welland Canal to 50% at a site off Nine Mile Point at the east end of Lake Ontario. By the fall of 1991, zebra mussel were present along the northwest end of Lake Ontario, while both zebra and quagga mussel were attached to buoys from the east end of the lake and in the St. Lawrence River.

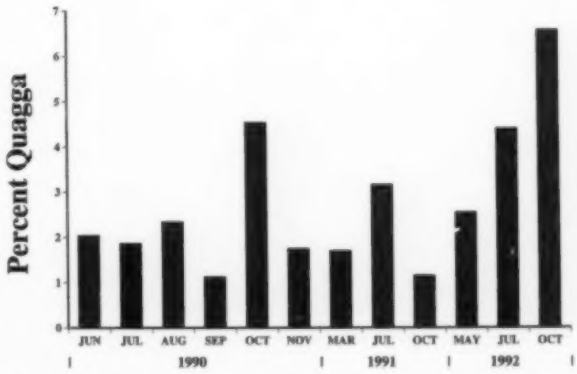



FIG. 2. Percent quagga mussel at the Port Colborne littoral site, 1990–92.

### Distribution of Quagga Mussel in 1992

Quagga mussel were identified at 25 of the 53 sites sampled in 1992 (Fig. 1). Locations with quaggas included Lake Erie sites at Van Buren Shoal, Brockton Shoal, Port Colborne, Port Stanley, and Nanticoke, Niagara River sites above and below Niagara Falls, all sites in Lake Ontario, and the St. Lawrence River. Quaggas were present in spring 1992 samples from Onondaga Lake, but were absent in fall collections of the same year. No quagga mussel were collected from Lake St. Clair, the Detroit River, western Lake Erie, the Erie–Barge Canal, Oneida Lake, the Mohawk River, the Hudson River, Cayuga Lake, and Seneca Lake, although these water bodies all contained zebra mussel.

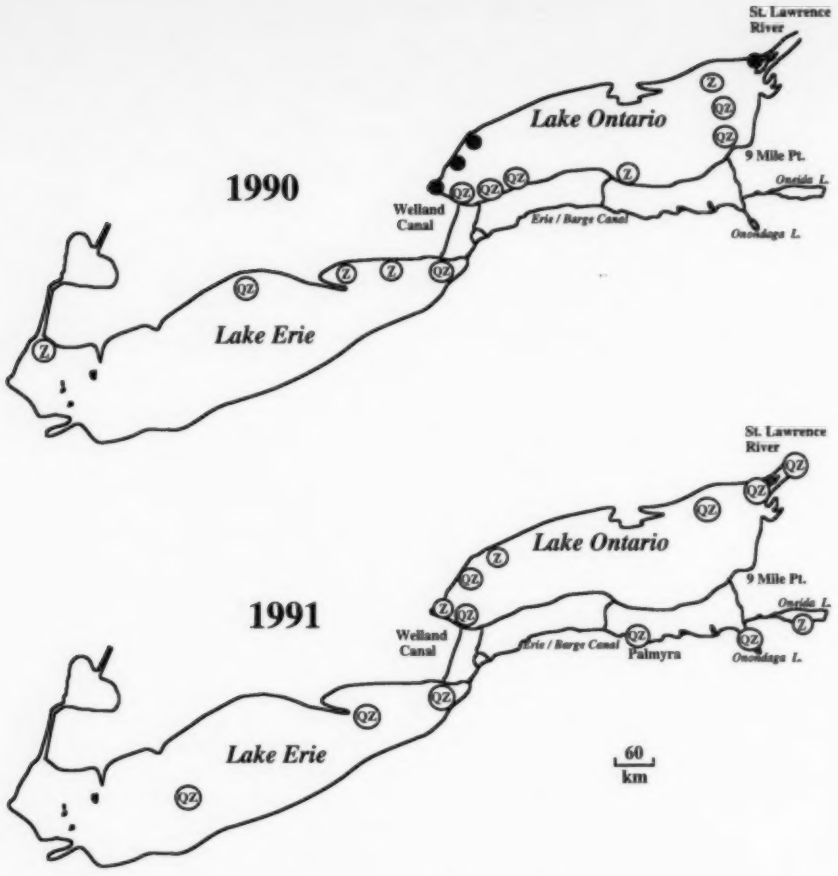



FIG. 3. Presence of quagga (Q) and zebra (Z) mussel and absence of both species (●) in samples from navigation buoys or Ponar grabs from the lower Great Lakes in 1990 and on buoys in 1991. Samples from the Erie–Barge Canal. Onondaga Lake, and Oneida Lake were collected by hand.

The remainder of the Finger Lakes (Conesus, Honeoye, Canandaigua, Keuka, Owasco, and Skaneateles) did not contain either quagga or zebra mussel. Zebra mussel were found at 47 of 53 sites, generally coexisting with quaggas except in the deeper offshore waters of Lake Ontario where only quaggas were found.

No quagga mussel were found on buoys retrieved during December 1992 from Lake St. Clair, the Detroit River, and Lake Erie west of Point Pelee. Both quagga and zebra mussel were present on all buoys ($N = 30$) retrieved from central and eastern Lake Erie. Proportions of quagga mussel ranged from 2.8% at Port Stanley in central Lake Erie to 94% of the mussels at Port Colborne in eastern Lake Erie (Fig. 1).

## Length–Frequency Distributions

Shells of quaggas were significantly larger than shells of zebra mussel at 7 of 11 sites (Table 2). Zebra mussel shells were significantly larger than those of quagga mussel at the Lake Erie sites (Table 2), but quaggas were larger than zebra mussel on most buoys in Lake Erie (Table 3). In addition, since the buoys are set out each spring (March), mussel size in the fall reflects the growth of individuals that settle on the buoys between spring and early fall. With the exception of Port Stanley, mean growth of quaggas was 1.2–1.6 times higher than that of zebra mussel on the buoys. The largest quagga and zebra mussel (quagga = 38 mm; zebra = 36 mm) were collected from Cape Vincent. In Lake Ontario, minimum shell size increased from west to east (i.e. Olcott to Cape Vincent).

Length–frequency distributions of quagga and zebra mussel differed between Lake Erie, the Niagara River, Lake Ontario, and the St. Lawrence River (Fig. 4). At the shallow site in eastern Lake Erie, shell lengths of quaggas were similar to those of zebra mussel and the length–frequencies of both dreissenids were skewed toward smaller shell lengths (2–14 mm). At Lake Ontario sites, shell lengths of zebra mussel were generally skewed toward smaller sizes whereas quagga shell lengths were larger and exhibited more normal distributions. In comparison, quagga lengths were skewed toward larger size categories both at the mouth of the St. Lawrence River at Cape Vincent (20–26 mm) and the lower reaches of the St. Lawrence River (26–36 mm) whereas most zebra mussel shell lengths in these two areas were in the smaller size categories (12–20 mm). Finally, quaggas were generally larger than zebra mussel except for the Lake Erie deep site where quaggas were smaller (Fig. 4).

The proportion of quaggas at two sites in Lake Ontario changed between spring and fall (Fig. 5). Proportions of quagga mussel were higher in the fall (chi-square, $p < 0.05$) at Olcott and

TABLE 2. Shell lengths of quagga (Q) and zebra (Z) mussel collected October to early November 1992 at sites where both species were present; for Lake Ontario sites, all depths were pooled (asterisk indicates significantly larger mean shell length; $N$ = sample size; SE = standard error).

| Site | Species | $N$ | Shell length (mm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Min | Max | $p$-value |
| Lake Erie (eastern basin) | | | | | | | |
| Shallow | Q | 201 | 8.6 | 0.335 | 3 | 29 | 0.0230[a] |
| | Z* | 891 | 9.4 | 0.108 | 3 | 20 | |
| Deep | Q | 393 | 10.1 | 0.245 | 3 | 20 | <0.0001[b] |
| | Z* | 29 | 17.4 | 0.977 | 7 | 27 | |
| Port Colborne | Q | 117 | 9.1 | 0.338 | 4 | 20 | <0.0001[b] |
| (littoral) | Z* | 116 | 11.8 | 0.303 | 5 | 21 | |
| Niagara River | Q* | 30 | 13.4 | 0.796 | 7 | 25 | 0.0002[a] |
| | Z | 354 | 9.9 | 0.178 | 4 | 19 | |
| Lake Ontario | | | | | | | |
| Olcott | Q* | 2020 | 12.6 | 0.123 | 2 | 30 | <0.0001[a] |
| | Z | 3547 | 10.8 | 0.075 | 2 | 25 | |
| Thirty Mile Point | Q* | 1849 | 14.0 | 0.124 | 4 | 32 | <0.0001[a] |
| | Z | 2873 | 9.9 | 0.086 | 2 | 22 | |
| Rochester | Q* | 293 | 12.8 | 0.238 | 4 | 32 | <0.0001[a] |
| | Z | 965 | 7.3 | 0.085 | 3 | 22 | |
| Smoky Point | Q* | 404 | 12.8 | 0.210 | 6 | 30 | <0.0001[b] |
| | Z | 840 | 4.0 | 0.081 | 3 | 19 | |
| Nine Mile Point | Q | 10 | 12.0 | 0.843 | 8 | 17 | 0.0740[a] |
| | Z | 467 | 10.3 | 0.131 | 3 | 25 | |
| Cape Vincent | Q* | 187 | 29.2 | 0.399 | 12 | 38 | <0.0001[b] |
| | Z | 1194 | 19.2 | 0.186 | 9 | 36 | |
| St. Lawrence River | Q* | 149 | 21.0 | 0.335 | 12 | 28 | <0.0001[a] |
| | Z | 1391 | 16.4 | 0.116 | 3 | 36 | |

[a] $t$-test.
[b] $G$-test.

TABLE 3. Shell lengths of quagga (Q) and zebra (Z) mussel collected from navigational buoys, November and December (asterisk indicates significantly larger mean shell length using the $t$-test; $N$ = sample size; SE = standard error).

| Site | Species | $N$ | Shell length (mm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mean | SE | Min | Max | $p$-value |
| Lake Erie | | | | | | | |
| Port Stanley | | | | | | | |
| Inshore | Q | 22 | 10.4 | 0.351 | 8 | 13 | <0.01[b] |
| | Z | 135 | 10.7 | 0.279 | 3 | 18 | |
| 3 km offshore | Q* | 39 | 9.5 | 0.259 | 5 | 12 | <0.0001[b] |
| | Z | 32 | 5.8 | 0.282 | 3 | 9 | |
| Nanticoke | | | | | | | |
| Inshore | Q* | 386 | 3.8 | 0.128 | 2 | 17 | 0.05[b] |
| | Z | 202 | 2.6 | 0.061 | 2 | 6 | |
| 8 km offshore | Q* | 133 | 5.1 | 0.166 | 2 | 14 | <0.0001[a] |
| | Z | 64 | 3.9 | 0.143 | 2 | 7 | |
| Port Colborne | | | | | | | |
| Inshore | Q* | 167 | 2.8 | 0.065 | 2 | 6 | <0.0001[b] |
| | Z | 11 | 2.3 | 0.141 | 2 | 3 | |
| 5 km offshore | Q* | 24 | 4.5 | 0.429 | 2 | 9 | <0.001[b] |
| | Z | 215 | 3.5 | 0.102 | 2 | 8 | |
| Lake Ontario, | | | | | | | |
| Port Weller | Q* | 33 | 6.6 | 0.359 | 2 | 9 | <0.0001[a] |
| | Z | 79 | 4.5 | 0.225 | 2 | 10 | |

[a] $t$-test.
[b] $G$-test.

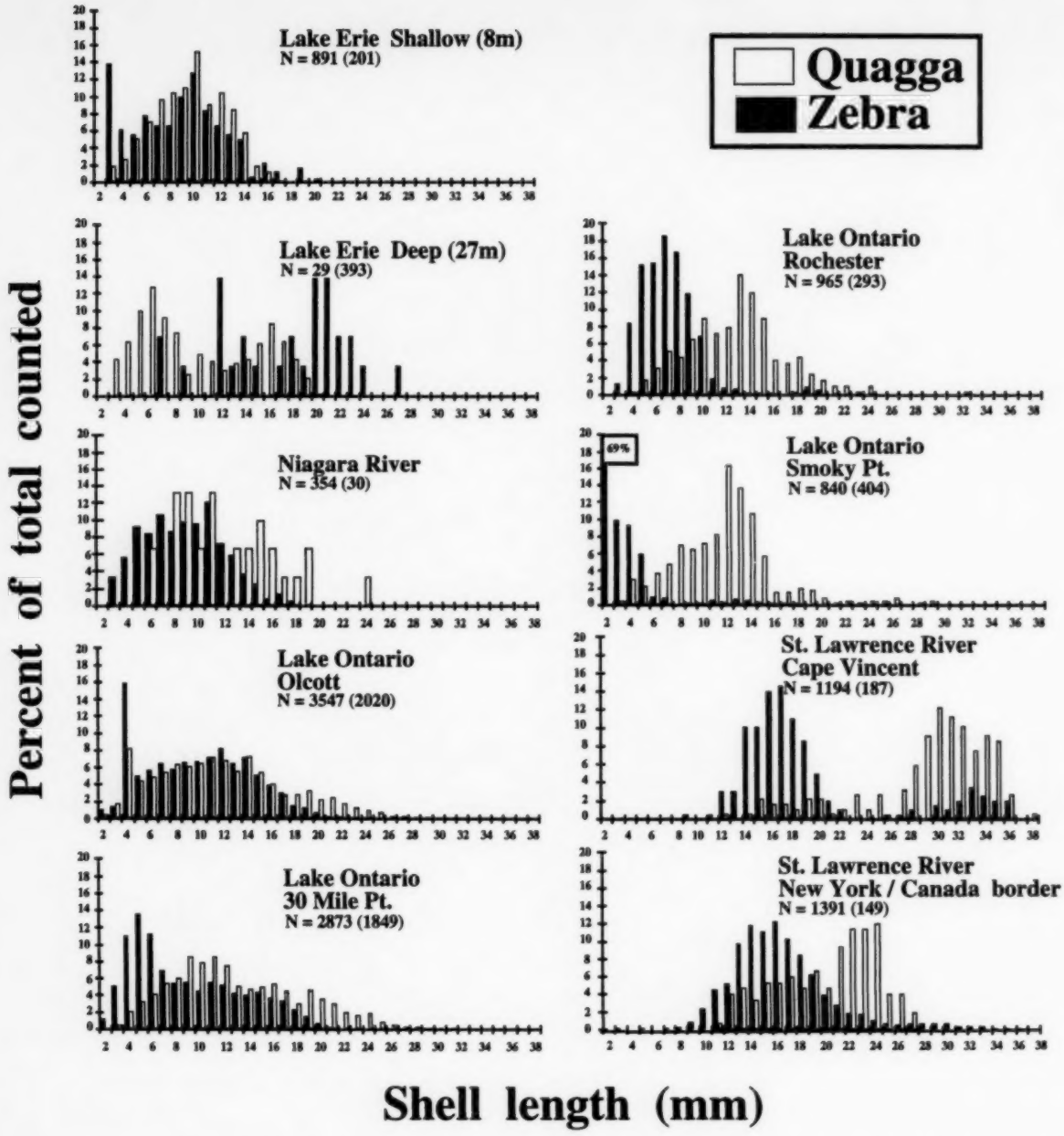



FIG. 4. Length–frequency distributions of quagga and zebra mussel from eastern Lake Erie, Lake Ontario, and the St. Lawrence River, October to early November 1992. *N* = number of zebra mussel (number of quagga mussel in parentheses).

Rochester compared with the spring. The increase in proportion of quaggas at Thirty Mile Point was not significant (chi-square, $p = 0.671$).

### Quagga Distribution, Lake Depth, and Bottom Water Temperature

Proportions of quagga mussel increased with lake depth in Lake Ontario in both the spring and fall (Fig. 6). At depths of >65 m, 60% or more of the mussel population were quaggas, the only exception being a sample composed of 33% quaggas at 85 m in spring. A sample of 100% quaggas was collected from a depth of 130 m. Quagga mussel also dominated the deeper waters of Lake Erie; in shallow waters (<8 m), 18% of the mussel population were quaggas whereas in deeper waters (27 m), 93% were quaggas.

Because of the apparent increase in proportion of quagga mussel in deeper parts of Lake Ontario, we examined the

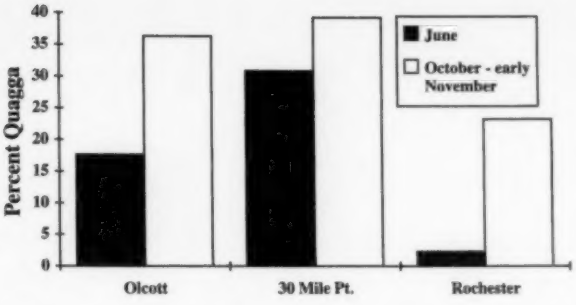



FIG. 5. Percent quagga mussel at sites along the south shore of Lake Ontario, June and October to early November 1992. Collections were made at the same depths using the same gear for both sampling periods.

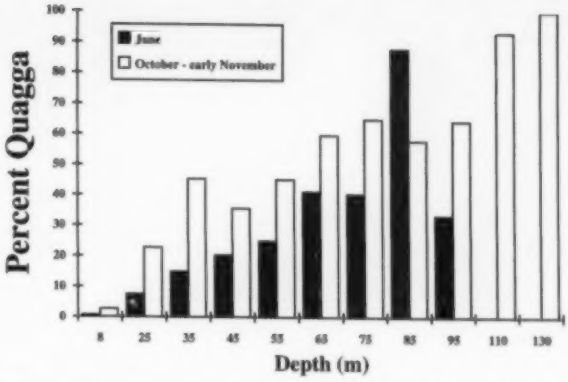



FIG. 6. Percent quagga mussel collected at different depths in Lake Ontario, June and October to early November 1992. For each depth, samples are pooled from Olcott, Thirty Mile Point, Rochester, and Smoky Point and no collections were made beyond 95 m in June.

relationship between lake depth and percent quaggas for all samples collected in the spring and fall (Table 4). The proportion of quagga mussel increased linearly with lake depth (Fig. 7). Lake bottom temperature covaried strongly with depth in Lake Ontario ($R^2 = 0.73$; $p < 0.001$); bottom water temperatures ranged from 3.5 to 8.4°C and decreased with lake depth (Table 4). As expected, the proportion of quagga mussel was negatively correlated with bottom water temperature. Using linear regression analysis, we found no significant relationship between mussel shell size and depth.

## Discussion

Quagga mussel abundance appears to exhibit an east to west gradient in Lake Erie with the heaviest colonization occurring in the eastern basin. By October 1991, quagga mussel had been collected in central Lake Erie, where they represented over 60% of the mussels attached to a buoy situated near the middle of the lake (Wormington et al. 1993). Further westward, quagga mussel became rare; in 1992, quaggas represented only 0.2% of the *Dreissena* on the north shore of the lake west of Rondeau (Dermott and Munawar 1993). D.W. Schloesser (USFWS, Ann Arbor, Mich., personal communication) has found quaggas comprising <1% of the mussel population in the western basin of Lake Erie as far west as Middle Sister Island. To date, there have been no reports of quagga mussel in the Great Lakes upstream of Lake Erie, although zebra mussel have established colonies at most Great Lakes ports (Griffiths et al. 1991). Quagga mussel are also present in the St. Lawrence River as far downstream as Montmagny, Quebec (L. Lapierre, St. Lawrence Centre, Environment Canada, personal communication).

Both *Dreissena* species were present in samples of the first mussels to settle along the south shore of Lake Ontario between the mouth of the Niagara River and Oswego during the summer and fall of 1990 (R. Dermott, unpublished data). Quagga mussel were also present among the first *Dreissena* to settle in eastern Lake Erie during the summer of 1989. Large quagga specimens (>28 mm) were collected during 1989 at Nanticoke, along the north shore of the eastern basin of Lake Erie (J.D. Ackerman, University of Toronto, Toronto, Ont., personal communication). Mackie (1991) reported zebra mussel growth rates in Lake St. Clair of between 1.5 and 2.0 cm during the first year of growth. If we assume quagga growth rates to be similar to those of zebra mussel, then the presence of large quaggas in 1989 suggests that this mussel was present in Lake Erie in 1988 and perhaps as early as 1987 when zebra mussel first invaded Lake Erie (Griffiths et al. 1991). However, if settlement of quagga and zebra mussel occurred at the same time on the navigational buoys sampled from Lakes Erie and Ontario (Table 3), then the greater shell lengths of quaggas relative to zebra mussel may indicate a faster growth rate for the quagga.

The depths at which we observed settled quagga and zebra mussel are among the deepest ever recorded. In Polish lakes, zebra mussel generally reach maximum densities between 2 and 4 m and tend to be sparse at depths >8 m (Stanczykowska 1977; Stanczykowska et al. 1983; Stanczykowska and Lewandowski 1993). Similar distributional patterns have been noted by other European researchers (e.g. Wesenberg-Lund 1939; Dunn 1954), although Walz (1973) found adult zebra mussel at a depth of 55 m and zebra mussel larvae at depths between 120 and 140 m in Lake Constance. In Lake Ontario, both quagga and zebra mussel coexist at depths of 8–110 m in varying proportions, with only quagga mussel being found at 130 m. In the eastern basin of Lake Erie, quaggas outnumbered zebra mussel by 14 to 1 in the deeper offshore waters and colonized soft substrates beyond 40 m (Dermott and Munawar 1993). These findings contrast sharply with European observations and raise new questions about the depths that these organisms can colonize in lakes.

In Lakes Ontario and Erie, the proportion of quagga mussel increases with depth and declines with increasing water temperature, suggesting that this mussel may be a cold deepwater form. In addition, quagga mussel were sparse or absent along inland waterways and lakes of New York State that were infested with zebra mussel. Low densities of quagga mussel were first reported from the Erie Canal in 1991 (May and Marsden 1992), yet we were unable to find quagga mussel in the Canal (at sites May and Marsden sampled) and at the outlet of Onondaga Lake in the fall of 1992. Why these sites are unsuitable for the persistance of quagga populations is unknown, but heavy infestations of zebra mussel competing for space and/or food could explain why quaggas appear to be excluded. In Lakes Erie and Ontario, the current spatial distribution of quaggas in relation to that of zebra mussel could also be a response to factors other than water temperature. For example, the quagga mussel could merely be occupying areas in Lakes Erie and Ontario which were not colonized earlier by zebra mussel. Quagga mussel, initially out-

TABLE 4. Average bottom water temperatures and mean shell sizes of quagga (Q) and zebra (Z) mussel collected at different depths in Lake Ontario, October to early November 1992. Mean, standard error (SE), and range of shell sizes are based on pooled samples from Olcott, Thirty Mile Point, Rochester, and Smoky Point sites.

| Depth (m) | Bottom water temp. (°C) | Species | *N* | Shell size (mm) | | |
|---|---|---|---|---|---|---|
| | | | | Mean | SE | Range |
| 25 | 8.4 | Q | 929 | 13.1 | 0.198 | 4–30 |
| | | Z | 3142 | 8.7 | 0.119 | 2–25 |
| 35 | 7.9 | Q | 1801 | 12.6 | 0.188 | 2–32 |
| | | Z | 2570 | 8.4 | 0.114 | 2–25 |
| 45 | 8.2 | Q | 1094 | 14.3 | 0.220 | 4–30 |
| | | Z | 1896 | 11.9 | 0.177 | 3–30 |
| 55 | 7.6 | Q | 325 | 12.7 | 0.188 | 2–25 |
| | | Z | 404 | 8.7 | 0.119 | 3–20 |
| 65 | 6.4 | Q | 86 | 12.5 | 0.188 | 4–25 |
| | | Z | 58 | 11.2 | 0.159 | 3–20 |
| 75 | 5.4 | Q | 156 | 13.2 | 0.200 | 4–23 |
| | | Z | 84 | 10.7 | 0.154 | 4–20 |
| 85 | 5.0 | Q | 107 | 13.7 | 0.208 | 5–23 |
| | | Z | 77 | 12.6 | 0.188 | 3–22 |
| 95 | 4.3 | Q | 63 | 11.4 | 0.167 | 3–22 |
| | | Z | 35 | 7.7 | 0.102 | 3–20 |
| 110 | 3.5 | Q | 14 | 15.1 | 0.235 | 6–24 |
| | | Z | 1 | 14.0 | | |
| 130 | 3.9 | Q | 5 | 14.0 | 0.214 | 10–19 |

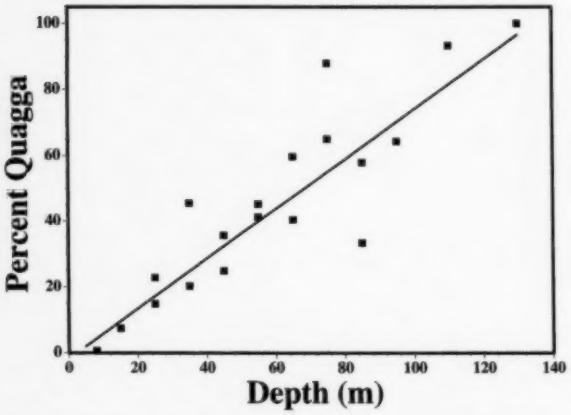



FIG. 7. Relationship between proportion of quagga mussel and depth at 19 different sites, June and October to early November 1992. Line fitted by regression equation: % quagga mussel = –0.42 + 0.770 (depth) (m); $R^2 = 0.794$; $p < 0.001$.

numbered by zebra mussel which became firmly established in nearshore waters, may simply be occupying deeper waters because of the availability of unused space. The lack of space may also be forcing both species to deeper waters. This argument is supported by the fact that zebra and quagga mussel coexist at depths to 110 m. On the other hand, quagga mussel may be poorly adapted morphologically to occupy nearshore depths which are subject to turbulence and wave action. Quaggas are flattened laterally, compared with the ventrally flattened zebra mussel; this allows the latter to become more firmly attached to hard bottom substrate materials and less prone to detachment (Morton 1993).

More intriguing is the apparent ability of both quagga and zebra mussel to survive at depths in Lake Ontario where temperatures rarely exceed 5°C (Saylor et al. 1981). If the mussels are able to successfully reproduce at these low temperatures, then this brings into question the 10–12°C threshold commonly reported for spawning and larval development (Sprung 1987; Borcherding 1991). The presence of newly settled mussels (<5 mm) at deepwater sites in Lake Ontario may indicate the possibility of successful recruitment at these low temperatures (Table 4). In addition, zebra mussel have been observed spawning at temperatures as low as 2.5°C (Walz 1978b). However, given an estimate of a zebra mussel's daily requirement of 75 µm carbon/mussel (Walz 1978a), it would appear that food rather than temperature would become the limiting factor at these depths. Starving mussels have been shown to become reproductively inhibited through the resorption of gonadal tissue in order to maintain vital physiological processes (Bielefeld 1991; Sprung and Borcherding 1991).

The assessment of population size structure given length–frequency distributions is confounded by factors such as variability in cohort growth rates, influence of water currents on growth rates, and decreased survival of juveniles in nearshore waters due to exposure and ice scour (Griffiths et al. 1991). Length–frequency distributions of quagga and zebra mussel from Lake Erie to the St. Lawrence River suggest that different environmental conditions are influencing population shell size structure. The average shell size of both quagga and zebra mussel ranged from 4 to nearly 30 mm and generally increased as one progressed from eastern Lake Erie to the St. Lawrence River.

In the shallow site in Lake Erie, length–frequency distributions of both dreissenids were skewed toward the smaller size categories (2–20 mm) (Fig. 4), suggesting that these populations were either food limited or that the absence of large mussel size categories was due to predation. French and Bur (1993) indicate

that freshwater drum (*Aplodinotus grunniens*), especially large adults, may reduce zebra mussel abundance in some habitats of western Lake Erie. However, because of the high fecundity of zebra mussel populations, it is unlikely that freshwater drum could suppress zebra mussel populations and control their population size structure. Alternatively, the reduced size of quagga and zebra mussel in Lake Erie compared with Lake Ontario sites may reflect intense competition for food and/or space. The average shell size of both mussels increased on the more isolated offshore buoys where competition for food would be lessened. The combination of high mussel densities in Lake Erie (more than 300 000/$m^2$; Leach 1993), their large collective filtering capacity (Kryger and Riisgard 1988), and reduced phytoplankton (MacIsaac et al. 1992) may not favor mussel growth. Compared with the shallow station, the Lake Erie deep site in Fig. 4 is very interesting because it shows size differences for the two dreissenid species which contrast all of the other histogram pairs. We can only speculate that this site might depict the westward migration of quaggas at the expense of zebra mussel (which show very little recruitment in comparison).

The pattern of shell size frequency for mussel populations in Lake Ontario differed from that observed in Lake Erie. In Lake Ontario, the two mussel species exhibited disparate shell size frequencies, as zebra mussel shell lengths were generally 2–12 mm whereas most quagga mussel were larger than 10 mm (Fig. 4). Such shell size segregation between the two dreissenid species may reflect density-dependent growth and the effects of food availability, water temperature, and depth. These factors have been shown to influence zebra mussel size in Polish lakes (Stanczykowska 1977). Based on the numbers of mussels collected in bottom trawls, we believe that zebra mussel populations are most abundant in the nearshore waters of Lake Ontario and decline as one proceeds offshore. Zebra mussel may be food limited in the nearshore waters of Lake Ontario whereas the increased shell size of quagga mussel offshore may reflect lower mussel densities and sufficient food availability to meet the demands for quagga growth.

Shell size frequencies of quagga and zebra mussel in the St. Lawrence River differed in comparison with other water bodies. Here, quaggas were larger than zebra mussel and there was little indication of smaller, newly recruited individuals of either species. Either recruitment in the upper sections of the river is occasional and limited to surplus additions from Lake Ontario or growth of cohorts is greater in flowing waters. Katchanova (1962) showed that growth of *Dreissena* in water channels was twice that seen in reservoirs, and Bij de Vaate (1991) found that growth of young-of-the-year mussels was accelerated in flowing waters. Another alternative is that currents in the upper sections of the river may be too swift to allow for mass larval settlement given that recruitment was found to increase in the St. Lawrence in those areas affected by tides (downstream of Trois Rivières, Quebec) (E. Mellina, unpublished data). The daily tidal cycle may afford larvae the opportunity to settle during those times when the incoming tides counteract the outgoing flow of water, resulting in negligible current speeds for short periods.

Dominance by quagga mussel in profundal areas indicates that the impacts of dreissenids on the food web will not be limited to shallow nearshore zones of large lakes. Colonization by quaggas could negatively impact profundal organisms like the burrowing amphipod *Diporeia* by competing for food and space (Dermott and Munawar 1993). The filtering and removal of food particles by quaggas could seriously deplete zooplankton food resources. Macroinvertebrates like *Mysis* also feed on zooplankton (Bowers and Vanderploeg 1982; Lasenby et al. 1986), so the indirect effects of the quagga mussel on zooplankton may ultimately have an impact on these organisms. On the other hand, colonization of profundal areas by quagga mussel could provide both a source of food from pseudofeces and a refugia in interstices of mussel colonies for macroinvertebrates like *Mysis*. *Mysis* and *Diporeia* are important food for planktivorous fish such as alewife (*Alosa pseudoharengus*) (Hewett and Stewart 1989; Mills et al. 1992), which in turn are critical food for salmonids (Eck and Brown 1985; Brandt 1986; Diana 1990; Elrod and O'Gorman 1991). Consequently, colonization of the profundal zone by the quagga mussel represents a new threat to the integrity of the Great Lakes and elevates the potential ecological significance of introduced mussels to North American freshwaters.

## Acknowledgements

This study was supported by funds from New York Sea Grant, Cornell University, St. Lawrence University, New York State Gas and Electric, Consolidated Edison, Rochester Gas and Electric, Central Hudson Gas and Electric, Monroe County Water Authority, and the Pew Charitable Trust. We especially thank Joseph H. Elrod, Randy Owens, Robert O'Gorman, the crew of the *Kaho*, and the U.S. Fish and Wildlife Service for allowing us to collect mussels during their routine spring and fall fish assessment surveys. We also thank Marty Horgan, Connie Adams, Don Bunnell, and two anonymous reviewers who made significant contributions to the manuscript. Contribution No. 153 of the Cornell Biological Field Station.

## References

Baker, F.C. 1902. The mollusca of Chicago area. Chicago Acad. Sci. Bull. 3(2): 1–410.

Baker, F.C. 1928. The American *Bithynia* not wholy an introduced species. Trans. Ill. State Acad. Sci. 20: 56–62.

Bielefeld, U. 1991. Histological observation of gonads and digestive gland in starving *Dreissena polymorpha* (Bivalvia). Malacologia 33: 31–42.

Bij de Vaate, A. 1991. Distribution and aspects of population dynamics of the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), in the Lake IJsselmeer area (The Netherlands). Oceologia 86: 40–50.

Borcherding, J. 1991. The annual reproductive cycle of the freshwater mussel *Dreissena polymorpha* Pallas in lakes. Oceologia 87: 208–218.

Bowers, J.A., and H.A. Vanderploeg. 1982. *In situ* predatory behavior of *Mysis relicta* in Lake Michigan. Hydrobiologia 93: 121–131.

Brandt, S.B. 1986. Food of trout and salmon in Lake Ontario. J. Great. Lakes Res. 12: 200–205.

Culligan, W.J., F.C. Cornelius, D.W. Einhouse, D.L. Zeller, and R.C. Zimar. 1992. 1992 Annual Report of the Bureau of Fisheries Lake Erie Unit to the Lake Erie Commission and the Great Lakes Fisheries Commission, April 1992. Publication of the New York State Department of Environmental Conservation. 23 p.

Dermott, R., and M. Munawar. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Can. J. Fish. Aquat. Sci. 50: 2298–2304.

Diana, J.S. 1990. Food habits of angler-caught salmonines in western Lake Huron. J. Great Lakes Res. 16: 271–278.

Domm, S., R.W. McCauley, E. Kott, and J.D. Ackerman. 1993. Physiological and taxonomic separation of two dreissenid mussels in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 50: 2294–2297.

Dunn, D.R. 1954. Notes on the bottom fauna of twelve Danish lakes. Vidensk. Medd. Dan. Naturhhist. Foren. 116: 251–268.

Eck, G.W., and E.H. Brown, Jr. 1985. Lake Michigan's capacity to support lake trout (*Salvelinus namayscush*) and other salmonines: an estimate based on the status of prey populations in the 1970's. Can. J. Fish. Aquat. Sci. 42: 449–454.

Elrod, J.H., and R. O'Gorman. 1991. Diet of juvenile lake trout in southern Lake Ontario in relation to abundance and size of prey fishes, 1979–1987. Trans. Am. Fish. Soc. 120: 290–302.

French, J.R.P., and M.T. Bur. 1993. Predation by the zebra mussel (*Dreissena polymorpha*) by freshwater drum in western Lake Erie, P. 453–464. *In* T.F.

Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

GRIFFITHS, R.W., D.W. SCHLOESSER, J.H. LEACH, AND W.P. KOVALAK. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Can. J. Fish. Aquat. Sci. 48: 1381–1388.

HAYES, M.L. 1983. Active fish capture techniques, p. 123-146. *In* L.A. Nielsen and D.L. Johnson [ed.] Fisheries techniques. American Fisheries Society, Bethesda, Md. 468 p.

HEWETT, S.J., AND D.J. STEWART. 1989. Zooplanktivory by alewives in Lake Michigan: ontogenic, seasonal, and historical patterns. Trans. Am. Fish. Soc. 118: 581–596.

KATCHANOVA, A.A. 1962. The ecology of *Dreissena polymorpha* in the Uchinsk reservoir. Vopr. Ekol. Vyssh. Shbala. 5: 94–95.

KRYGER, J., AND H.U. RIISGARD. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. Oecologia 77: 34–38.

LASENBY, D.C., T.G. NORTHCOTE, AND M. FURST. 1986. Theory, practice, and effects of *Mysis relicta* on introductions to North American and Scandinavian lakes. Can. J. Fish. Aquat. Sci. 43: 1277–1284.

LEACH, J.H. 1993. Impacts of the zebra mussel, *Dreissena polymorpha*, on water quality and spawning reefs in western Lake Erie, p. 381–398. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

LUDYANSKIY, M.L. 1991. Are *Dreissena polymorpha* and *Dreissena bugensis* synonymous? *Dreissena polymorpha* Inf. Rev. N.Y. Sea Grant.

LUDYANSKIY, M.L. 1993. Recent introductions of *Dreissena* and other forms into North American — the Caspian Sea/Black Sea connection, p. 699–704. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

MACISAAC, H.J., W.G. SPRULES, O.E. JOHANNSSON, AND J.H. LEACH. 1992. Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Oecologia 92: 30–39.

MARELLI, D. 1991, Zhadin's systematic account of *Dreissena*: are the species valid? *Dreissena polymorpha* Inf. Rev. N.Y. Sea Grant 2(4).

MARELLI, D.C., AND S. GRAY. 1985. Comments on the status of recent members of the genus *Mytilopsis* (Bivalvia: Dreissenidae). Malacol. Rev. 18: 117–122.

MAY, B., AND J.E. MARSDEN. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. 49: 1501–1506.

MILLS, E.L., J.H. LEACH, J.T. CARLTON, AND C.L. SECOR. 1993. Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. J. Great Lakes Res. 19: 1–54.

MILLS, E.L., R. O'GORMAN, J. DEGISI, R.F. HEBERGER, AND R.A. HOUSE. 1992. Food of the alewife (*Alosa pseudoharengus*) in Lake Ontario before and after the establishment of *Bythotrephes cederstroemi*. Can. J. Fish. Aquat. Sci. 49: 2009–2019.

MORTON, B. 1993. The anatomy of *Dreissena polymorpha* and the evolution and success of the heteromyarian form in the Dreissenoidea, p. 185–215. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

NUTTALL, C.P. 1990. Review of the Caenozoic heterodont bivalve superfamily Dreissenacea. Palaeontology 33: 707–737.

O'GORMAN, R., E.L. MILLS, AND J.S. DEGISI. 1991. Use of zooplankton to assess the movement and distribution of alewife (*Alosa pseudoharengus*) in south-central Lake Ontario in spring. Can. J. Fish. Aquat. Sci. 48: 2250–2257.

SAYLOR, J.H., J.R. BENNETT, P.C. LIU, R.L. PICKETT, F.M. BOYCE, C.R. MURTHY, AND T.J. SIMONS. 1981. Water movements, p. 247–292. *In* E.J. Aubert and T.L. Richards [ed.] The International Field Year for the Great Lakes. NOAA, Ann Arbor, Mich.

SNEDECOR, G.W., AND W.C. COCHRAN. 1980. Statistical methods. Iowa State University Press, Ames, Iowa. 593 p.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., New York, N.Y. 859 p.

SPRUNG, M. 1987. Ecological requirements of developing *Dreissena polymorpha* eggs. Arch. Hydrobiol. 79(Suppl.): 69–86.

SPRUNG, M., AND J. BORCHERDING. 1991. Physiological and morphometric changes in *Dreissena polymorpha* (Mollusca: Bivalvia) during a starvation period. Malacologia 33: 179–191.

STANCZYKOWSKA, A. 1977. Ecology of *Dreissena polymorpha* (Pallas) (Bivalvia) in lakes. Pol. Arch. Hydrobiol. 24: 461–530.

STANCZYKOWSKA, A., K. LEWANDOWSKI, AND J. EJSMONT-KARABIN. 1983. Biotic structure and processes in the Lake system of R. Jorka watershed (Mazurian Lakeland, Poland). IX. Occurrence and distribution of molluscs with special consideration to *Dreissena polymorpha* (Pall.). Ekol. Pol. 31: 761–780.

STANCZYKOWSKA, A., AND K. LEWANDOWSKI. 1993. Thirty years of studies of *Dreissena polymorpha* in Mazurian Lakes of northeastern Poland, p. 3–33. *In* T.F. Nalepa and D.W. Schloesser [ed.] Zebra mussels: biology, impacts, and control. Lewis Publishers, Inc., Boca Raton, Fla. 810 p.

WALZ, N. 1973. Studies on the biology of *Dreissena polymorpha* Pallas in the Lake of Constance. Arch. Hydrobiol. 42(Suppl.): 452–482.

WALZ, N. 1978a. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. I. Pattern of activity, feeding and assimilation efficiency. Arch. Hydrobiol. 55(Suppl.): 83–105.

WALZ, N. 1978b. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. II. Reproduction. Arch. Hydrobiol. 55(Suppl.): 106–119.

WESENBERG-LUND, C. 1939. Biologie der Susswassertiere. Springer-Verlag, Wien, Germany. 817 p.

WORMINGTON, A., C.A. TIMMINS, AND R.M. DERMOTT. 1993. Distribution of zebra mussels on Canadian navigation buoys on the Great Lakes and Upper St. Lawrence River, December 1992. Can. MS. Rep. Fish. Aquat. Sci. 2186: 35 p.

ZHADIN, V.I. 1952. Mollusks of fresh and brackish waters of the U.S.S.R.: keys to the fauna of the U.S.S.R. Zool. Inst. Izd. Akad. Nauk S.S.S.R. Moskva-Leningrad 46: 376 p.

# Effect of Changes in Recruitment Levels on Multispecies Long-term Predictions

Henrik Gislason

*Danish Institute for Fisheries and Marine Research, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark*

Gislason, H. 1993. Effect of changes in recruitment levels on multispecies long-term predictions. Can. J. Fish. Aquat. Sci. 50: 2315–2322.

A multispecies model (MSFOR) is used to predict the relative change in equilibrium yield and spawning stock biomass (SSB) of commercially important fish stocks in the North Sea resulting from a reduction in the fishing mortality generated by the roundfish fishery. Because predation mortality is a function of the abundance of prey and predators the results will depend on recruitment. Assuming recruitment to be independent of stock sizes the effect of changes in recruitment is studied by repeating the predictions at all possible combinations of ±50% changes in predator and prey recruitment levels. All of the predictions result in a relative increase in the SSB of Atlantic cod (*Gadus morhua*) and saithe (*Pollachius virens*) and in a relative decrease in the SSB of Atlantic herring (*Clupea harengus*) and Norway pout (*Trisopterus esmarkii*). In these cases the sign of the relative change is robust to recruitment changes. However, for haddock (*Melanogrammus aeglefinus*), sprat (*Sprattus sprattus*), and sandeel (*Ammodytes marinus*) the relative change in SSB is found to be either positive or negative depending on the level of recruitment. The predictions for haddock are highly sensitive to changes in the level of saithe recruitment.

Un modèle plurispécifique (MSFOR) est utilisé pour prévoir le changement relatif du rendement à l'équilibre et de la biomasse du stock reproducteur de stocks de poissons de grande valeur commerciale dans le mer du Nord dû à une réduction de la mortalité par pêche produite par la pêche des poissons ronds. Comme la mortalité par prédation est fonction de l'abondance des proies et des prédateurs, les résultats dépendront du recrutement. En supposant que le recrutement est indépendant de la taille du stock, l'effet des variations du recrutement est étudié en répétant les prévisions pour toutes les combinaisons possibles de variation de ± 50 % du niveau de recrutement chez les prédateurs et chez les proies. Toutes les prévisions se traduisent par une augmentation relative de la biomasse du stock reproducteur de la morue franche (*Gadus morhua*) et de la goberge (*Pollachius virens*) et une baisse relative de la biomasse du stock reproducteur du hareng de l'Atlantique (*Clupea harengus*) et du tacaud norvégien (*Trisopterus esmarkii*). Dans ces cas, le signe du changement relatif est robuste par rapport aux modifications du recrutement. Toutefois, dans le cas de l'aiglefin (*Melanogrammus aeglefinus*), du sprat (*Sprattus sprattus*) et du lançon (*Ammodytes marinus*), le changement relatif de la biomasse du stock reproducteur est positif ou négatif selon le niveau de recrutement. Les prévisions pour l'aiglefin sont très sensibles aux variations du niveau de recrutement de la goberge.

*Received May 4, 1992*
*Accepted June 23, 1993*
*(JB485)*

*Reçu le 4 mai 1992*
*Accepté le 23 juin 1993*

The prediction of recruitment to fish stocks is a central problem in fisheries management. Although many have attempted to model recruitment, their success has been limited (Wooster and Bailey 1989). For this reason, yield predictions have been made using models for which the effect of the fishery can be examined independently of the effects of changes in recruitment levels.

One particularly successful approach has been the development of single-species yield-per-recruit models, such as those of Thompson and Bell (1934) and Beverton and Holt (1957). Assuming that growth and natural mortality remain constant over time, these models describe the average yield obtained from each recruit that enters the stock for a given exploitation pattern. The total yield from the population is then obtained by multiplying the yield-per-recruit by the expected number of recruits. If recruitment is assumed to be independent of spawning stock size the yield-per-recruit curve reflects the relative change in total yield. If this assumption is not valid, a suitable stock–recruitment relationship must be introduced.

In multispecies models incorporating technical and biological interactions, recruitment is no longer a simple multiplier and the effect of the fishery cannot easily be separated from the recruitment problem. In models incorporating technical interaction (Murawski 1984; Sainsbury 1984; Pikitch 1987; Murawski et al. 1991), where several species or stocks are exploited by the same fishing fleet, it is necessary to predict the recruitment to each species relative to the recruitment to the other species exploited by the fleet in order to estimate the relative change in total yield. In models where species interaction is included, growth and natural mortality may vary as a function of the density of prey and predators. In the multispecies VPA (MSVPA) and corresponding forecast (MSFOR) model (Helgason and Gislason 1979; Gislason and Helgason 1985; Gislason and Sparre 1987; Sparre 1991), growth is assumed constant, but predation mortality is directly proportional to the number of predators and inversely proportional to the total amount of prey available to these predators. Predation mortality and yield-per-recruit will therefore depend on recruitment to the prey and predator stocks

included in the model (Gislason 1991). In multispecies predictions, it is therefore much more difficult to examine the effect of the fishery independent of the recruitment problem.

In this paper, I have used the MSFOR to predict the outcome of a 25% reduction in effort in the North Sea roundfish fishery. I have expressed the results as percent changes in yield and biomass and evaluated how various changes in the level of recruitment would affect the predictions. Assuming recruitment to be restricted to within ±50% of the present average, my results show that definite statements about the sign of the relative changes in yield and biomass can be made, provided recruitment is independent of stock sizes.

## MSVPA and MSFOR Models and Database for the North Sea

The MSVPA and MSFOR (Helgason and Gislason 1979; Gislason and Helgason 1985; Gislason and Sparre 1987; Sparre 1991) are multispecies extensions to the single-species VPA (Gulland 1965) and forecast model (Thompson and Bell 1934) in which biological interaction due to predation is taken into account. The MSVPA utilizes catch-at-age and food composition data to estimate fishing mortality, predation mortality, and stock sizes. The MSFOR is the predictive counterpart of the MSVPA and includes both technical and biological interaction. Given estimates of future recruitment and fishing mortality the MSFOR can be used to predict long-term yield and biomass.

The basic equations of the MSVPA and MSFOR are the same as in the single-species model:

$$N_{i,t+1} = N_{i,t}\, e^{-Z_{i,t}}$$

$$C_{i,t} = \frac{F_i N_{i,t} (1 - e^{-Z_{i,t}})}{Z_{i,t}}$$

$$Z_{i,t} = F_{i,t} + M_{i,t}$$

where $N_{i,t}$ is the stock size of species age group $i$ at time $t$, $C_{i,t}$ is the catch of $i$ during the time interval from $t$ to $t + 1$, $Z_{i,t}$ is the total mortality, $F_{i,t}$ is the fishing mortality, and $M_{i,t}$ is the total natural mortality.

However, in addition, the models include species interaction due to predation. Predation mortality is entered by dividing the total natural mortality, $M_{i,t}$, into mortality due to predation and mortality due to all other natural cases. Thus:

$$M_{i,t} = M1_i + M2_{i,t}$$

where $M2_{i,t}$ is the predation mortality generated by the predators included in the model and $M1_i$ is a residual mortality assumed to be constant. Assuming growth and total food intake per capita at age to be constant over time, the predation mortality, $M2_{i,t}$, is estimated from

$$M2_{i,t} = \sum_p \frac{S_{i,p} R_p \overline{N_{p,t}}}{\overline{N_{i,t}}\, W_i}$$

where the summation runs over all predator species age groups $p$, $S_{i,p}$ is the fraction of the food on a weight basis of predator species age group $p$ which consists of prey species age group $i$, $R_p$ is the total food intake (per individual and time interval) of $p$, $W_i$ is the weight of prey species age group $i$ at ingestion, and $\overline{N_{i,t}}$ is the average stock size of $i$ in the time interval between $t$ and $t + 1$:

$$\overline{N_{i,t}} = \frac{N_{i,t}(1 - e^{-Z_{i,t}})}{Z_{i,t}}.$$

The food composition, $S_{i,p}$, is made a function of prey abundance by an equation developed by Andersen and Ursin (1977). In the model, $S_{i,p}$ is estimated as the biomass of prey species age group $i$ weighted by the suitability of $i$ as food for $p$ divided by the sum of the biomass of all potential prey items weighted by their respective suitabilities:

$$S_{i,p} = \frac{G_{i,p} \overline{N_{i,t}}\, W_i}{\sum_k G_{k,p} \overline{N_{k,t}}\, W_k + G_{o,p} B_o}$$

where $G_{i,p}$ is the suitability of prey species age group $i$ to predator species age group $p$, $k$ is the total number of prey species age groups, and $B_o$ is the biomass of "other food," $o$, i.e., food consisting of species not directly included in the model. The biomass of other food and the suitabilities are assumed to remain constant over time.

As shown by Sparre (1980) the above equation can be rearranged to

$$G_{i,p} = \frac{\frac{S_{i,p}}{\overline{N_{i,t}}\, W_i}}{\sum_k \frac{S_{k,p}}{\overline{N_{k,t}}\, W_k} + \frac{S_{o,p}}{B_o}}.$$

Provided historic data on food composition are available, this equation can be used to estimate the suitabilities within the MSVPA (Gislason and Sparre 1987; Sparre 1991).

The input to the MSVPA consists of catch in numbers at age, food composition, total food intake per capita, weight-at-age, and fishing mortality for the oldest age group in each cohort. Given these data the equations describing catch, stock numbers, and predation mortalities are solved simultaneously for all predator and prey stocks within a given time interval. As in the single-species VPA the estimated stock numbers are used as input to the calculations for the preceding time interval. Because the calculations are only carried back to 1 July for the 0-group, the model does not account for predation on eggs, larvae, and small juveniles. The output from the MSVPA consists of estimates of stock sizes, fishing mortalities, predation mortalities, and suitability coefficients.

The MSFOR is based on the same equations as the MSVPA. The suitabilities, total food intake per capita, weight-at-age, and terminal stock sizes are usually transferred directly from the MSVPA to the MSFOR. Given fishing mortality and recruitment, the MSFOR can be used to estimate the long-term equilibrium yield in weight as well as the total biomass and spawning stock biomass (SSB). The total fishery may be subdivided into a number of fleets. Given the proportion of the total fishing mortality on each species and age group generated by each of the fleets as well as estimates of the weight-at-age in the catch by

fleet, the model is able to predict the future yield in weight by fleet. If data on the value per kilogram at age are available, it is also possible to obtain an estimate of the total landed value. Recruitment may be assumed either to be constant at some arbitrary level (e.g. historic average) or to be a stochastic process in which year class strength is drawn at random from lognormal distributions around the average level. If recruitment is assumed to be constant, the calculations continue until an equilibrium is reached in which the change in stock size over a 9-yr period is less than 0.01%. This usually requires predicting approximately 50 yr ahead in time. In the case of stochastic recruitment the model continues for a user-specified number of years (e.g., 2000) after which the means and standard deviations of the generated time series of yield and biomass are estimated.

The MSVPA and MSFOR models have been used over a number of years by the International Council for the Exploration of the Sea (ICES) Multispecies Assessment Working Group to assess the fisheries in the North Sea (Pope 1991). The results presented here are based on the same input data as those used at the 1990 and 1992 meetings of the Working Group (Anonymous 1991, 1992). These data include 11 species of which five are predators (Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), and Atlantic mackerel (*Scomber scombrus*)), seven are prey (Atlantic cod, whiting, haddock, Atlantic herring (*Clupea harengus*), sprat (*Sprattus sprattus*), Norway pout (*Trisopterus esmarkii*), and sandeel (*Ammodytes marinus*)), and two (sole (*Solea solea*) and plaice (*Pleuronectes platessa*)) do not interact with the others. The catch-at-age data and terminal fishing mortalities needed in the MSVPA have been derived from ICES Assessment Working Group reports. Estimates of the food composition of the five predators are based on the stomach content data collected during the ICES stomach sampling project (Anonymous 1988; Daan 1989). In the MSFOR the North Sea fishery is divided into seven fleets (roundfish, industrial demersal, industrial pelagic, saithe, Atlantic mackerel, Atlantic herring, and flatfish). The roundfish fleet catches Atlantic cod, whiting, saithe, and haddock. Because part of the haddock and whiting catches are discarded, the fishing mortality in the roundfish fleet is divided into a landings mortality and a discard mortality. The industrial demersal fleet catches Norway pout, sandeel, haddock, saithe, and whiting, and the industrial pelagic fleet catches Atlantic herring, sprat, and whiting. The Atlantic herring, Atlantic mackerel, and saithe fisheries are single-species fisheries, while the flatfish fishery catches both plaice and sole.

## MSFOR and Changes in Recruitment

Recruitment changes take place over various temporal scales, from year-to-year variations to changes that span several decades.

The effect of year-to-year changes in recruitment was studied by Gislason (1991) who showed that the average yield and biomass from MSFOR predictions in which year class strength was drawn at random from lognormal distributions were identical to the equilibrium yield and biomass obtained with constant recruitment. The conclusion was that even though species interaction is modeled by nonlinear equations in the MSFOR, this nonlinearity does not appear to be strong enough to generate a difference between the results from stochastic and constant recruitment runs. This is comforting, as it signifies that it is possible to neglect year-to-year variations in the predictions leaving only the effect of longer term changes in the average level of recruitment to be considered.

In the North Sea, several species have been subject to changes in the level of recruitment during the period for which reliable data exists. The so-called "gadoid outburst," in which a number of extraordinary year classes of Atlantic cod, whiting, and haddock gave rise to a substantial increase in stock size of all three species, lasted from the start of the 1960s to the mid-1970s (Cushing 1982). North Sea Atlantic mackerel have suffered from very low recruitment since 1971, and the North Sea Atlantic herring stocks showed a marked drop in recruitment from the mid-1970s to the first half of the 1980s (Daan et al. 1990).

Gislason (1991) showed that changes in the level of recruitment for a particular species resulted directly in changes in the yield-per-recruit predicted by the MSFOR. For most species, however, changes in recruitment did not seem to affect the conclusions regarding the sign of the change in yield or biomass following a change in the level of fishing mortality. Only for Atlantic cod were the conclusions dependent on recruitment. At high levels of recruitment the highest yield-per-recruit was obtained when fishing mortality was increased above the present level. At low levels of recruitment, the highest yield-per-recruit was found at levels of fishing mortality lower than the present. This result was attributed to cannibalism. At low levels of stock size (low recruitment), cannibalism is relatively unimportant compared with growth and the stock is in a state characterized by growth overfishing. In this situation, the highest yield-per-recruit is obtained if fishing mortality is reduced. At high levels of stock size (high recruitment), mortality due to cannibalism becomes important, and in this situation the highest yield-per-recruit is obtained when fishing mortality is increased. The general conclusion was, therefore, that multispecies long-term predictions of relative gains and losses depend on the level of future recruitment. The paper considered, however, only how the yield-per-recruit of a particular species changed as a function of recruitment to the species itself. The effect of changes in recruitment for other species was not considered.

In order to study the influence of changes in recruitment levels for a number of species, I extended the MSFOR program with a routine that estimates the percent difference in yield and biomass between two long-term predictions at different combinations of recruitment levels. I then used the program to evaluate the relative change in long-term yield and biomass between a status quo prediction, in which the fishing mortality was kept at the average 1984–88 level, and a prediction in which the fishing mortality generated by the roundfish fleet was reduced by 25% compared with the status quo situation. For convenience, relative rather than absolute differences were compared. The reason for this choice is that relative differences between two fishing strategies are likely to be less sensitive to changes in recruitment level than absolute differences. In order to investigate the sensitivity to recruitment changes, the difference between the two strategies was estimated at various combinations of ±50% of the average historic recruitment levels for individual species estimated by the MSVPA for the period 1974–88. A factorial design in which all combinations of high and low recruitment levels for each of the 11 stocks are covered would require a total of $2^{11}$ comparisons. However, because sole and plaice do not interact with other species and are caught only by the flatfish fleet, the results for these two species would not be affected by changes in roundfish effort. The recruitment for sole and plaice was

TABLE 1. Percent change in long-term equilibrium landings and discards from the roundfish fleet following a 25% reduction in the fishing mortality generated by this fleet. Recruitment kept constant at the average 1974–88 level.

| Species | Landings | Discards |
|---|---|---|
| Atlantic cod | −8 | 0 |
| Whiting | −11 | −25 |
| Saithe | −9 | 0 |
| Haddock | −16 | −37 |
| Total | −11 | −33 |

therefore kept constant at the average 1984–88 level. With two levels for each of the remaining nine interacting species included in the model, a total of $2^9$ (512) combinations of levels is possible. For each combination, the percent difference between the status quo situation and a 25% reduction in roundfish effort was estimated. On a HP9000 series 730 workstation, one run consisting of 1024 predictions takes approximately half an hour. The results are presented as tables of the percent difference in yield and SSB for each of the 512 comparisons.

## Results

Tables 1 and 2 show a comparison between the status quo prediction and the 25% reduction in roundfish effort when recruitment to all species is kept constant at the average 1974–88 level. Table 1 shows the percent difference in yield from the roundfish fleet. The largest percent change is seen in the discards of whiting and haddock that decrease by 25 and 37%, respectively. All landings are predicted to decrease. Table 2 shows the percent changes in total biomass, SSB, and total catch (including discards). The SSBs of Atlantic cod, whiting, haddock, and saithe are predicted to increase, while the SSBs of Atlantic herring and Norway pout decrease. For Atlantic cod the SSB will increase by 61%; for the other species the changes are smaller.

The results shown in Tables 1 and 2 have all been generated under the assumption that recruitment remained at a level identical to the average recruitment in the period from 1974 to 1988. An additional run, in which all combinations of high and low recruitment to each of the nine interacting species were tested, was used to investigate the effect of changes in recruitment level. For each combination of recruitment levels, the long-term (equilibrium) percent difference between the status quo situation and the 25% reduction in the effort of the roundfish fleet was estimated.

Table 3 shows the frequency distribution of the percent change in landings and discards by species in the roundfish fleet. Out of the 512 comparisons, 27.3% resulted in a 10–20% reduction, in the long-term landings of Atlantic cod, 66.2% in a 0.01–10% reduction, and 6.4% in a 0.01–10% increase. The conclusion is, therefore, that the predicted 8% decrease in Atlantic cod landings (see Table 1) that was made under the assumption that recruitment remained at the average 1974–88 level is sensitive to changes in recruitment level. In 6.4% of the investigated cases, the predicted loss was actually converted into an increase in landings. In the case of whiting, the landings from the roundfish fleet are predicted to decrease between 0.01 and 20% and the discards between 20 and 30%. Saithe, which is not preyed upon in the model and for which the traditional single-species yield-per-recruit model may be applied, is, as expected, insensitive to

TABLE 2. Percent change in long-term equilibrium biomass, SSB, and total North Sea catch predicted by the MSFOR following a 25% reduction in the fishing mortality generated by the roundfish fleet. Recruitment kept constant at the average 1974–88 level.

| Species | Total biomass | SSB | Catch |
|---|---|---|---|
| Atlantic cod | 21 | 61 | −8 |
| Whiting | 2 | 3 | −11 |
| Saithe | 13 | 34 | 3 |
| Atlantic mackerel | 0 | 0 | 0 |
| Haddock | −8 | 4 | −24 |
| Atlantic herring | −5 | −6 | −7 |
| Sprat | 0 | 0 | 0 |
| Norway pout | −8 | −12 | −21 |
| Sandeel | 0 | 0 | 0 |
| Plaice | 0 | 0 | 0 |
| Sole | 0 | 0 | 0 |
| Total | −1 | 0 | −7 |

changes in recruitment level. All runs result in a 9% decrease in saithe landings. Haddock, on the other hand, is extremely sensitive to changes in recruitment level. Within the levels of recruitment defined above, the results range from a 50–60% reduction to a 0.01–10% increase in landings and from a 20 to a 70% decrease in discards.

The distribution of the percent change in SSB is shown in Table 4. For Atlantic cod, whiting, and saithe, a relative increase in SSB is expected in all cases. The SSBs of Atlantic herring and Norway pout show a relative decrease for all combinations of recruitment levels, while the reduction in the fishing effort of the roundfish fleet may result either in a relative decrease or in a relative increase in SSBs of haddock, sandeel, and sprat. Sole and plaice are not caught by the roundfish fleet and do not serve as prey for any of the five predators. They are therefore unaffected by the effort reduction.

Both in terms of landings and SSB, the distribution of the percent change for haddock is surprisingly wide, and it would be interesting to know whether this is caused by changes in the level of recruitment to the haddock stock itself or by changes in recruitment to the other stocks, i.e., the predators of haddock and/or their alternative prey. In order to investigate this question further, an ANOVA was performed in which the percent changes in landings, discards, and SSBs were considered as the response variables and the recruitment levels as classification variables. The ANOVA explains the variation in percent change as due to differences in recruitment level with random error to account for all remaining variation:

$$y_j = \alpha_j + \sum_m \beta_m + \varepsilon_j$$

where the summation runs over all species, $\alpha_j$ is the percent change for species $j$ if recruitment is at a high level for all species, $\beta_m$ is the effect of low recruitment for species, $m$, and $\varepsilon_j$ is the random error term.

The parameter estimates are shown in Table 5, where the intercept, i.e., −9.7% for Atlantic cod, may be interpreted as the expected percent change in landings resulting from the reduction in effort in the roundfish fleet if recruitment is at a high level for all species. The other parameters show the effect of a change in recruitment level from a high to a low level. They may be added

TABLE 3. Distribution of percent long-term changes in landings and discards from the North Sea roundfish fleet subject to changes in levels of recruitment. Status quo compared with 25% reduction in fishing mortality. Results from 512 comparisons.

| | Landings | | | | Discards | |
|---|---|---|---|---|---|---|
| % change | Atlantic cod | Whiting | Saithe | Haddock | Whiting | Haddock |
| 60 to 70 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 to 60 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 to 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 to 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 to 30 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 to 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.01 to 10 | 6.4 | 0 | 0 | 4.3 | 0 | 0 |
| −0.01 to 0.01 | 0 | 0 | 0 | 0 | 0 | 0 |
| −10 to −0.01 | 66.2 | 46.3 | 100.0 | 34.2 | 0 | 0 |
| −20 to −10 | 27.3 | 53.7 | 0 | 19.7 | 0 | 0 |
| −30 to −20 | 0 | 0 | 0 | 16.8 | 100.0 | 25.8 |
| −40 to −50 | 0 | 0 | 0 | 13.3 | 0 | 33.4 |
| −50 to −60 | 0 | 0 | 0 | 11.7 | 0 | 18.8 |
| −60 to −70 | 0 | 0 | 0 | 0 | 0 | 22.1 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

TABLE 4. Distribution of percent long-term changes in spawning stock biomass subject to changes in levels of recruitment. Status quo compared with 25% reduction in the fishing mortality generated by the North Sea roundfish fleet. Results from 512 comparisons.

| % change | Atlantic cod | Whiting | Saithe | Atlantic mackerel | Haddock | Atlantic herring | Sprat | Norway pout | Sandeel | All species |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 to 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 70 to 80 | 24.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 to 70 | 33.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 to 60 | 36.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 to 50 | 6.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 to 40 | 0 | 0 | 100.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 to 30 | 0 | 0 | 0 | 0 | 16.0 | 0 | 0 | 0 | 0 | 0 |
| 10 to 20 | 0 | 0 | 0 | 0 | 27.5 | 0 | 0 | 0 | 0 | 0 |
| 0.01 to 10 | 0 | 100.0 | 0 | 0 | 13.7 | 0 | 21.5 | 0 | 30.1 | 53.7 |
| −0.01 to 0.01 | 0 | 0 | 0 | 100.0 | 0 | 0 | 1.6 | 0 | 1.0 | 0 |
| −10 to −0.01 | 0 | 0 | 0 | 0 | 17.4 | 94.1 | 77.0 | 48.8 | 68.9 | 46.3 |
| −20 to −10 | 0 | 0 | 0 | 0 | 3.9 | 5.9 | 0 | 51.2 | 0 | 0 |
| −30 to −20 | 0 | 0 | 0 | 0 | 19.7 | 0 | 0 | 0 | 0 | 0 |
| −40 to −50 | 0 | 0 | 0 | 0 | 1.8 | 0 | 0 | 0 | 0 | 0 |
| −50 to −60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| −60 to −70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| −70 to −80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| −80 to −90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

to the intercept in order to estimate the expected response. Consider, for example, the situation where Atlantic cod recruitment is at a low level and recruitment to all other stocks is at a high level. The expected percent change in landings of Atlantic cod can then be estimated as $-9.7 + 6.8 = -2.9\%$. The parameters thus summarize both the expected direction and the magnitude of the percent change in landings in light of changes in the level of recruitment.

The only situation in which a decrease in roundfish effort may result in a relative increase in Atlantic cod landings is when the recruitments to both saithe and Atlantic cod are at a low level. In this situation the mortality due to cannibalism by Atlantic cod and predation by saithe is reduced to a level where the present level of fishing mortality results in growth overfishing. In all other cases the percent change will be negative irrespective of recruitment level. However, the change in haddock landings may be positive if saithe recruitment is at a low level. The percent changes in haddock landings and discards are very sensitive to changes in the level of recruitment of saithe and Norway pout. When recruitment of saithe is at a low level the natural mortality of haddock is reduced. In this situation, growth overfishing is possible, in which case the landings will increase when fishing mortality is reduced.

The results of the ANOVA of SSB are given in Table 6. Saithe, Atlantic mackerel, plaice, and sole, for which a change in recruitment level does not change the percent increase/decrease in SSB, have not been included. The predicted changes in SSB for Atlantic cod and whiting are most sensitive to changes in the

TABLE 5. Results of an ANOVA of the percent change in landings and discards from the roundfish fleet upon a 25% reduction in the fishing mortality generated by the North Sea roundfish fleet at high and low levels of recruitment. The intercept is the expected change if all recruitment are at a high level.

| | Landings | | | Discards | |
|---|---|---|---|---|---|
| Parameter | Atlantic cod | Whiting | Haddock | Whiting | Haddock |
| Intercept | −9.7 | −11.8 | −24.0 | −25.6 | −41.9 |
| Recruitment low for: | | | | | |
| Atlantic cod | 6.8 | 4.0 | 2.7 | 0.7 | 0.3 |
| Whiting | −0.2 | 0.3 | 2.9 | 0.5 | 1.9 |
| Saithe | 4.4 | 1.3 | 24.3 | 0.8 | 16.2 |
| Atlantic mackerel | −0.1 | 0.1 | 0.4 | 0.6 | 0.2 |
| Haddock | −0.8 | −0.4 | −1.8 | −0.1 | −0.9 |
| Atlantic herring | −1.1 | −0.4 | −1.2 | −0.1 | −0.7 |
| Sprat | −0.2 | −0.2 | −0.7 | −0.1 | −0.4 |
| Norway pout | −2.9 | −1.0 | −12.5 | −0.6 | −8.0 |
| Sandeel | −0.8 | −0.7 | −2.7 | −0.3 | −1.5 |
| $R^2$ | 0.99 | 0.97 | 0.96 | 0.93 | 0.96 |

TABLE 6. Results of an ANOVA of the percent change in spawning stock biomass upon a 25% reduction in the fishing mortality generated by the North Sea roundfish fleet at high and low levels of recruitment. The intercept is the expected change if all recruitment are at a high level. Only species for which a change in recruitment level produces a change in the percent increase/decrease of SSB have been included.

| Parameter | Atlantic cod | Whiting | Haddock | Atlantic herring | Sprat | Norway pout | Sandeel |
|---|---|---|---|---|---|---|---|
| Intercept | 57.1 | 2.9 | −5.3 | −6.9 | −0.6 | −11.9 | 0.3 |
| Recruitment low for | | | | | | | |
| Atlantic cod | 13.4 | 2.0 | 3.1 | −1.2 | −0.5 | −0.6 | −0.9 |
| Whiting | −0.4 | 0.6 | 3.6 | 1.0 | −0.8 | 0.1 | −0.8 |
| Saithe | 7.7 | 1.4 | 30.1 | 2.5 | 0.3 | 5.0 | −0.4 |
| Atlantic mackerel | −0.1 | 0.1 | 0.5 | 0.0 | −0.0 | −0.0 | −0.1 |
| Haddock | −1.3 | −0.3 | −2.2 | −0.1 | 0.2 | 0.1 | −0.4 |
| Atlantic herring | −2.0 | −0.2 | −1.4 | −0.4 | 0.1 | −0.1 | 0.3 |
| Sprat | −0.4 | −0.2 | −0.9 | −0.2 | 0.3 | −0.0 | 0.1 |
| Norway pout | −5.0 | −1.0 | −15.4 | −1.6 | 0.5 | −0.8 | 0.6 |
| Sandeel | −1.3 | −0.6 | −3.4 | 0.3 | 0.1 | −0.3 | −0.1 |
| $R^2$ | 0.99 | 0.96 | 0.96 | 0.95 | 0.53 | 0.80 | 0.52 |

level of recruitment to the species themselves. For haddock, the results show that the very wide range of predicted change in SSB, i.e., the large spread of values in Table 4, is due mainly to changes in the recruitment level of saithe and Norway pout. Changing the recruitment level of whiting from a high to a low level would add 3.6% to the predicted change in SSB, while a similar decrease in saithe recruitment would add 30.1%. These results point to the importance of saithe as a predator and stress the significance of improving the estimates of food composition and stock size for saithe in particular.

The correlation $R^2$ expresses the proportion of the total variance that is explained by the analysis. Because there is one observation per cell and only main effects are included in the ANOVA, $R^2$ may also be interpreted as a measure of the importance of second- and higher-order interactions. With the exception of sprat and sandeel SSBs, the effects of changes in recruitment level on the percent change in catch and SSB seem to be well explained by the main effects ($R^2 > 0.9$). In the case of sprat and sandeel SSBs, $R^2$ is lower; thus for these species, second-order interactions should be taken into account.

## Discussion

In the multispecies calculations presented here, recruitment has been restricted to ±50% of the historic average; within these limits it has not been further specified. However, as in the single-species yield-per-recruit model, the results can only be interpreted as directly reflecting the relative changes in absolute yield and biomass if recruitment is assumed to be independent of stock size.

If recruitment depends on stock size, a change in exploitation pattern and subsequent stock size may influence recruitment, resulting in misleading comparisons of two different exploitation patterns at a constant level of recruitment. In this case the MSFOR cannot be used to predict the relative changes in absolute yield and stock size without incorporating a set of suitable

stock–recruitment relationships.

If, on the other hand, recruitment is assumed to be independent of stock size within a range of ±50% of the present level the 512 comparisons can be interpreted as reflecting the limits of the relative change in absolute yield and biomass. In a multispecies context, however, this independence signifies not only that recruitment is independent of the size of the parent stock, but also that it is not affected by changes in the size of other stocks included in the model (i.e., potential competitors and predators influencing the survival of larvae and juveniles prior to recruitment).

For the commercially exploited fish stocks in the North Sea, it seems reasonable to assume that recruitment is independent of spawning stock size, provided the latter is kept within certain limits. Daan et al. (1990) analyzed the historic time series and concluded that no correlation was present between SSB and subsequent recruitment for North Sea stocks, with the possible exception of Atlantic herring and Atlantic mackerel. Provided recruitment in the North Sea is independent of stock size and remains within ±50% of the present level, the results for Atlantic cod, whiting, Atlantic herring, and Norway pout show that definite conclusions about the sign of a long-term percent change in SSB, landings, and discards can be reached, even though the future level of recruitment is uncertain. For other species, notably haddock, the results show that not even the direction of the long-term changes can be predicted. However, these conclusions depend on whether the arbitrarily selected recruitment limits of ±50% of the present average are sensible. MSFOR predictions are based on the assumptions of constant growth and constant residual mortality. These assumptions, and the assumption that recruitment is independent of stock size, require that stock size (or spawning stock size) is kept within certain limits. The problem is to define the corresponding maximum and minimum levels of recruitment.

The Advisory Committee for Fisheries Management (ACFM), which meets under the auspices of ICES, has the task of providing biological advice to the managers of the North Sea fisheries. The advice given by ACFM is partly based on the so-called "minimum biologically acceptable level" (MBAL) of SSB (ACFM 1991). In stocks for which there is adequate historic information, this level has been defined, in the single-species context, from plots of recruitment versus SSB from which is inferred a level of SSB below which the probability of poor recruitment increases as spawning stock decreases. In stocks for which information is limited, MBAL is assumed to correspond to the lowest level of spawning stock so far recorded. Stocks below this level are in "unknown territory"; therefore, any action resulting in a spawning stock below this level would be outside safe biological limits, even though the actual level of SSB below which the probability of poor recruitment increases is unknown. The MBAL approach is limited not only by lack of data, but also by our limited understanding of the effects of the environment on the survival of larval and juvenile fish prior to recruitment. Given these conditions, it is almost impossible to predict how stock–recruitment relationships will be affected in the future. Most likely, MBAL will change over time.

At present, the SSB is below MBAL for a number of stocks. The SSB of North Sea Atlantic mackerel has been below MBAL since the beginning of the 1970s when the last large year classes were produced. The spawning stock sizes of Atlantic cod and haddock are presently the lowest on record and below the minimum levels indicated by ACFM (1992). In these cases, it is quite possible that future recruitment is dependent on changes in SSB. In situations where one exploitation pattern results in SSB predictions above the minimum level and the other in estimates below it, the estimates of the relative change in long-term yield and biomass cannot be trusted. The predictions for Atlantic cod and haddock should therefore be treated with utmost caution. Continued fishing at the present level of effort could result in a collapse of the fishery in both cases.

Sensible upper limits above which the assumptions of constant growth, residual mortality, and density-independent recruitment are likely to be violated have not previously been defined. As an approximation to these limits, the maximum stock sizes observed in the period for which reliable data exist may be used. The drawback of this approach is, again, that it is uncertain whether these stock sizes can be realized with the present rate of exploitation and environmental and ecological conditions. At the very least the maximum stock sizes should be corrected for changes in exploitation.

Instead of an a priori definition of upper and lower levels of recruitment, an alternative, and perhaps more fruitful, approach could be to determine the recruitment interval within which the model would produce the same conclusions regarding a change in exploitation pattern as a prediction with recruitment constant at the historic average. This would correspond to a curve in the response space for which the relative difference between the two fishing strategies would be zero. Such an approach would, however, not make the discussion of upper and lower levels redundant. It would still be necessary to establish to what extent the assumptions behind the model were fulfilled.

Apart from the definition of upper and lower limits of recruitment, another, albeit minor, problem that deserves further attention is the possibility of local maxima and minima of the response within the appropriate recruitment space. Until now the response has only been estimated at all possible combinations of high and low levels of recruitment, i.e., only at the corners of the recruitment space. If, as in the technical interaction case, the response is a linear function of recruitment, theory tells us that the maximum and minimum values of the response are found at the corners. If, as in the species interaction case, the response is a nonlinear function of recruitment, a sufficient number of recruitment levels should be examined in order to verify that all comparisons fall within the distribution already obtained.

The main purpose of the present paper has been to demonstrate that conclusions that are independent of recruitment may be drawn from multispecies predictions. It is fortunate that this is possible, as it will allow fisheries biologists to develop a multispecies framework for providing management advice that is robust to changes in recruitment not only in the short term, but also in the long term where changes in recruitment level could be expected to have a considerable impact.

## Acknowledgments

The ICES stomach sampling programme and the development of multispecies assessment within the ICES Multispecies Assessment Working Group have involved a large number of people at all stages between collection of material and construction of multispecies computer programmes. It is impossible to mention all of these people individually, but the present paper would not have been possible without the integrated efforts of many scientists over a number of years. At the Danish Institute for Fisheries and Marine Research, I thank Katherine Richardson, Hans Lassen, Per Sparre, Eskild Kirkegård, and several others for useful suggestions on an early draft and Leif Thomsen for help with the computer programme. The constructive comments of

Niels Daan and an anonymous reviewer considerably improved the manuscript. Financial support was received from the European Community through the Community research programme for the fisheries sector, FAR (contract No. MA 2 556).

## References

ACFM. 1991. Reports of the Advisory Committee on Fishery Management. ICES Coop. Res. Rep. No. 173.

ACFM. 1992. Reports of the Advisory Committee on Fishery Management. ICES Coop. Res. Rep. No. 179.

ANDERSEN, K.P., AND E. URSIN. 1977. A multispecies extension to the Beverton and Holt theory of fishing, with accounts of phosphorus circulation and primary production. Meddr Dan. Fisk. Havunders. N.S. 7: 319–435.

ANONYMOUS. 1988. Report of the meeting of the coordinators in the stomach sampling programme 1985 to 1987. ICES CM 1988/G:27.

ANONYMOUS. 1991. Report of the Multispecies Assessment Working Group. ICES CM 1991/Assess:7.

ANONYMOUS. 1992. Report of the Multispecies Assessment Working Group. ICES CM 1992/Assess:16.

BEVERTON, R.J.H., AND S.J. HOLT. 1957. On the dynamics of exploited fish populations. Fish. Invest. Lond. Ser. 2, 19: 533 p.

CUSHING, D.H. 1982. The decline of the herring stocks and the gadoid outburst. J. Const. Int. Explor. Mer 39: 70–81.

DAAN, N. [ED.] 1989. Data base report of the stomach sampling project 1981. ICES Coop. Res. Rep. No. 164: 144 p.

DAAN, N., P.J. BROMLEY, J.R.G. HISLOP, AND N.A. NIELSEN. 1990. Ecology of North Sea fish. Neth. J. Sea Res. 26(2–4): 343–386.

GISLASON, H. 1991. The influence of variations in recruitment on multispecies yield predictions in the North Sea. ICES Mar. Sci. Symp. 193: 50–59.

GISLASON, H., AND T. HELGASON. 1985. Species interaction in assessment of fish stocks with special application to the North Sea. Dana 5: 1–44.

GISLASON, H., AND P. SPARRE. 1987. Some theoretical aspects of the implementation of multispecies virtual population analysis in ICES. ICES CM 1987/G:51.

GULLAND, J.A. 1965. Estimation of mortality rates. Annex to Arctic Fisheries Working Group report. ICES CM 1965/3.

HELGASON, T., AND H. GISLASON. 1979. VPA-analysis with species interaction due to predation. ICES CM 1979/G:52.

MURAWSKI, S.A. 1984. Mixed-species yield-per-recruitment analysis accounting for technological interactions. Can. J. Fish. Aquat. Sci. 41: 897–916.

MURAWSKI, S.A., A.M. LANGE, AND J.S. IDOINE. 1991. An analysis of technological interactions among Gulf of Maine mixed-species fisheries. ICES Mar. Sci. Symp. 193: 237–252.

PIKITCH, E.K. 1987. Use of a mixed-species yield-per-recruit model to explore the consequences of various management policies for the Oregon flatfish fishery. Can. J. Fish. Aquat. Sci. 44(Suppl. 2): 349–359.

POPE, J.G. 1991. The ICES Multispecies Assessment Working Group: evolution insights, and future problems. ICES Mar. Sci. Symp. 193: 22–33.

SAINSBURY, K.J. 1984. Optimal mesh size for tropical multispecies trawl fisheries. J. Cons. Int. Explor. Mer. 44: 189–199.

SPARRE, P. 1980. A goal function of fisheries (Legion analysis). ICES CM 1980/G:40.

SPARRE, P. 1991. Introduction to multispecies virtual population analysis. ICES Mar. Sci. Symp. 193: 12–21.

THOMPSON, W.F., AND F.H. BELL. 1934. Biological statistics in the Pacific halibut fishery. 2. Effect of changes in intensity upon total yield and yield per unit of gear. Rep. Int. Fish. (Pac. Halibut) Comm. 8: 49 p.

WOOSTER, W.S., AND K.M. BAILEY. 1989. Recruitment of marine fishes revisited, p. 153–159. *In* R.J. Beamish and G.A. McFarlane [ed.] Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. Can. Spec. Publ. Fish. Aquat. Sci. 108.

# An Index of the Relative Abundance of Wintering Belugas, *Delphinapterus leucas*, and Narwhals, *Monodon monoceros*, off West Greenland

M.P. Heide-Jørgensen, H. Lassen, and J. Teilmann

*Marine Mammal Section, Greenland Fisheries Research Institute, Tagensvej 135, DK-2200 Copenhagen N, Denmark*

and R.A. Davis

*LGL Environmental Research Associates, 22 Fisher Street, PO Box 280, King City, ON L0G 1K0, Canada*

Heide-Jørgensen, M.P., H. Lassen, J. Teilmann, and R.A. Davis. 1993. An index of the relative abundance of wintering belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, off West Greenland. Can. J. Fish. Aquat. Sci. 50: 2323–2335.

Systematic aerial surveys of the wintering grounds of belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, in southern Baffin Bay and northern Davis Strait were conducted in late winter of 1981, 1982, 1990, and 1991. Most belugas were found between 67°N and 69°N and none were seen more than 80 km off the coast of West Greenland. Compared with the surveys in 1981 and 1982, a decline in relative abundance of belugas along West Greenland was evident in 1991. This decline was significant at a probability level of 0.13 of the bootstrapped distribution of the combined abundance estimate. Pod sizes declined significantly between the 1981–82 and 1990–91 surveys. The variations in ice conditions between years did not seem to affect the distribution, clumping, or pod sizes of the belugas. Narwhals were widely distributed in the close pack ice offshore between 65°N and 72°N. Along the West Greenland coast, narwhals were primarily seen at the mouth of Disko Bay. No change in relative abundance or pod sizes could be detected for narwhals.

Des relevés aériens systématiques des aires d'hivernage du béluga, *Delphinapterus leucas*, et du narval, *Monodon monoceros*, dans le sud de la baie Baffin et le nord du détroit de Davis, ont été effectuées à la fin de l'hiver de 1981, 1982, 1990 et 1991. La plupart des bélugas se trouvaient entre le 67°N et le 69°N et aucun n'a été observé à plus de 80 km de la côte ouest du Groenland. Par rapport aux relevés de 1981 et de 1982, on a enregistré, en 1991, une baisse évidente de l'abondance relative du béluga le long de la côte ouest du Groenland. Cette baisse était significative pour un niveau de probabilité de 0,13 de la distribution de l'estimation combinée de l'abondance obtenue par la méthode « bootstrap ». La taille des troupeaux a diminué considérablement entre les relevés de 1981–1982 et ceux de 1990–1991. Les variations de l'état des glaces d'une année à l'autre n'a pas semblé modifier la distribution, le regroupement et la taille des troupeaux de bélugas. Les narvals étaient largement répartis sur la banquise serrée entre le 65°N et le 72°N. Le long de la côte ouest du Groenland, les narvals étaient surtout observés à l'entrée de la baie Disko. Chez le narval, aucune modification de l'abondance relative ou de la taille des troupeaux n'a pu être décelée.

*Received July 30, 1992*
*Accepted May 21, 1993*
*(JB583)*

*Reçu le 30 juillet 1992*
*Accepté le 21 mai 1993*

Belugas or white whales, *Delphinapterus leucas*, are widely distributed in Baffin Bay. From December through May, they are found along the West Greenland coast from about 66°N to 70°N where they are particularly abundant in the area bounded by 67–69°N and 54–55°W. This West Greenland area probably constitutes the main wintering area of the Canadian High Arctic stock although some belugas winter in the North Water along southeastern Devon Island and in eastern Jones Sound and Smith Sound (Vibe 1950; Freeman 1968; Finley and Renaud 1980). According to the catch statistics, belugas are rarely caught in Greenland north of 70°N during winter months (Kapel 1977). During summer months from late June through mid-September, belugas from West Greenland probably concentrate in the Canadian High Arctic where large congregations are found in some of the bays adjacent to Lancaster Sound and Barrow Strait (Sergeant and Brodie 1975; Smith et al. 1985; Reeves and Mitchell 1987). No tagging or genetic studies have so far confirmed the relationship between West Greenland and Canadian High Arctic belugas, but the timing and direction of the movements suggest an autumn migration from Canada towards West Greenland (Reeves and Mitchell 1987). Apparently, most belugas leave Greenlandic waters during the spring (May–June) and they are only occasionally taken in Avanersuaq District (77°N, 70°W) in July–August. In late September, large herds are sometimes seen and hunted in Avanersuaq, and from early October through November, large numbers are usually taken in Upernavik District (74°N, 57°W; Heide-Jørgensen 1994). They are caught throughout the winter along West Greenland and in Disko Bay. In spring, belugas are taken on their northward migration from the ice edges of Uummannaq, Upernavik, and Avanersuaq districts.

Baffin Bay belugas have been subject to considerable exploitation, particularly in Greenlandic waters where the average annual catch was estimated at about 900 whales during the late

1980's (Heide-Jørgensen 1994). Overexploitation before 1930 probably caused their disappearance from the southern part of their range south of 65°N (Degerbøl and Nielsen 1930; Reeves and Mitchell 1987; Heide-Jørgensen 1994).

The abundance of belugas on the Canadian summering grounds was assessed from aerial surveys in 1981 at 12 450 whales (95% CI: 6300 – 18 600; Smith et al. 1985). However, this represents a minimum estimate of abundance because survey coverage did not include parts of the summer range including Peel and Jones sounds and because counts were not corrected for submerged whales. However, no estimator is yet available for application to aerial surveys of Baffin Bay belugas to account for the submerged proportion of the population. Only their relative abundance can be estimated.

Unlike belugas, narwhals, *Monodon monoceros*, are widely distributed in the close pack ice in northern Davis Strait and southern Baffin Bay during winter months, and some narwhals evidently also winter in the North Water (Finley and Renaud 1980; McLaren and Davis 1983). In West Greenland, they are caught as far south as 66°N in late winter, but most catches are made at the southern entrance to Disko Bay (Kapel 1977; Heide-Jørgensen 1994). Along the fast ice edge in Uummannaq and Upernavik, narwhals are often caught in spring during a northward movement, presumably to their summering grounds in Avanersuaq District and the Canadian High Arctic. In late autumn, a southward movement is first observed in Upernavik and later in Uummannaq (Heide-Jørgensen 1994).

The abundance of narwhals in the Canadian High Arctic was estimated at 17 900 (95% CI: 13 100 – 21 400) in 1984 from an aerial photographic survey (Stong 1988). A simultaneous land-based count in Avanersuaq provided an estimate of 4000 narwhals (Born 1986). Narwhals summering in Melville Bay and Smith Sound were not censused, nor was the estimate corrected to account for whales that were submerged during the suveys.

In West Greenland the first censuses of belugas, narwhals, and other marine mammals were conducted in March 1981 and 1982 (McLaren and Davis 1983). To ascertain whether population sizes had changed since the 1981 and 1982 surveys, we decided to repeat the surveys in 1990 and 1991 and to conduct comparable analysis of survey results from all years.

## Material and Methods

### Survey Design

Systematic aerial surveys of marine mammals in southern Baffin Bay and central Davis Strait were conducted in 1981, 1982, 1990, and 1991, but timing and survey procedures varied slightly among years (Fig. 1; Table 1). In 1981 and 1982 the surveys were flown in a deHavilland Twin Otter (CF-ARC) in which observations of animals and of sea and ice conditions were made from the right front (copilot's) seat and the left side two seats behind the pilot. Sightings and environmental conditions were recorded at 2-min intervals on portable tape recorders to facilitate later determination of the positions of observations. In 1990 and 1991, a Partenavia P-68 Observer (OY-CAG) aircraft equipped with Plexiglas bubble windows on both rear windows was used for the aerial surveys. Observations of animals were made from each of the bubble windows, while observations on weather and ice conditions were made by the pilot. Data on both sightings and environmental conditions were collected in 2-min segments. The two observers and the pilot recorded their observations on three independent tape recorders using hand-held microphones. When activated, the tape recorders received a synchronized time signal from an Epson HX-100 computer.

Strip widths were determined using inclinometers (Table 1). The transect width was 1600 m in 1981 and 1982, but because of the blind angle below the aircraft, an effective transect width of 1400 m was used for the density calculations. With the bubble windows used in 1990 and 1991, it was possible to observe animals on the trackline straight below the aircraft. In 1990 the width of the transect on each side of the plane was divided into an inner and outer transect of 400 m. In 1991 the perpendicular angle to all whale sightings was measured by inclinometer to permit determination of transect width a posteriori. Distance of the observation from the trackline was calculated from

$$(1)\quad \text{Distance} = \text{altitude}\cdot\tan(90 - \text{angle})$$

where angle to the whale was measured when passing abeam.

A pod was defined as two or more whales exhibiting the same behavior (e.g., swimming direction) or being within two to three body lengths of each other. We attempted to classify belugas into broad age groups based on their color and size. Three age groups were recognized: white, gray, and newborns. Gray animals accompanying adults were classified as calves.

Visibility, sea state, and ice conditions were recorded. Sea state was classified using the Beaufort Scale. Surveys were terminated when sea states exceeded Beaufort Scale 3. The ice coverage on the transects was subjectively assessed by the pilot or one of the observers. In 1981, six ice classifications were used but they were not compatible with the classifications used subsequently. In 1982, 1990, and 1991 the ice was classified in tenths. Real-time information on ice conditions was also obtained from NOAA satellite images provided by the Danish Meteorological Institute.

In 1981 and 1982, both north–south transects separated by 15° longitude and east–west transects separated by 15 nautical miles (M) were flown (Fig. 1). In 1990 and 1991, we flew east–west transects separated by 10 M in 1990 and by 5 M in 1991 (Fig. 1). Transit time between transects was used for resting the observers. The same observers were used for the surveys in 1981 and 1982 whereas two new observers were used in 1990 and 1991.

### Statistical Analysis

The surveyed area was divided into five strata (Fig. 2), and the number of whales observed on each transect was the sampling unit.

The surveys in 1981 and 1982 were systematic but with transects of unequal lengths. Some covered the strata in a north–south direction and some in an east–west direction. The surveys in 1990 and 1991 had parallel transects of equal lengths separated by 5 and 10 M, respectively, in rectangular strata (Figs. 1 and 2).

Estimates of abundance and the appropriate analytical variances were calculated by the ratio method developed by Cochran (1977) and its modifications for transects of equal length (Caughley 1977; Caughley and Grigg 1981) and for systematic surveys with serial correlation of densities between neighboring transects (Kingsley et al. 1985).

In addition and for the comparison of the overall abundance between years, we also calculated the estimate of the total number of surfaced whales, $p$, in a stratum as

$$(2)\quad p = [\Sigma A\cdot n_j / e_j]/K = \text{mean}(p_j)$$

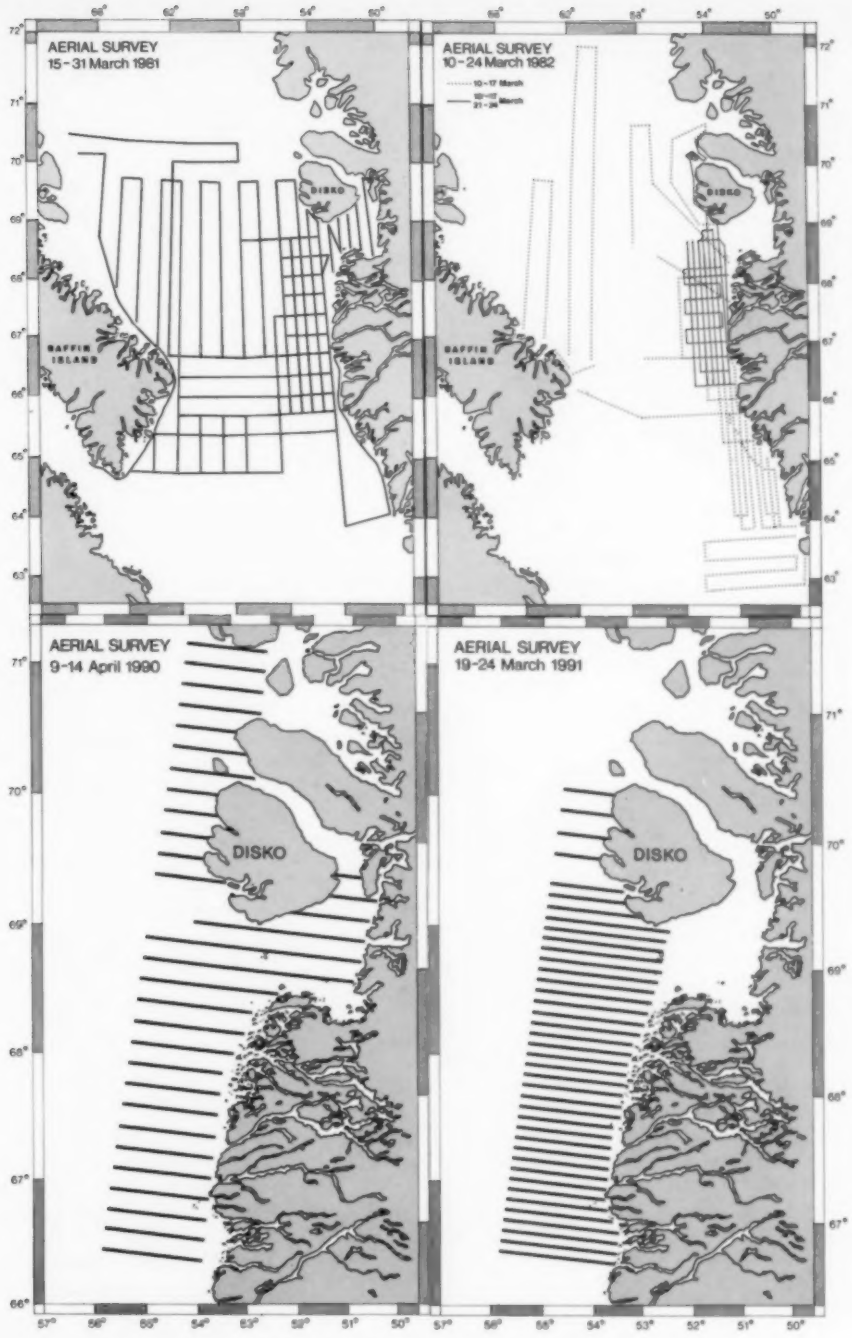



FIG. 1. Survey routes flown over northern Davis Strait and southern Baffin Bay in 1981, 1982, 1990, and 1991. In 1982 the dotted routes were flown on 10–17 March, while the routes off central West Greenland were covered twice: 10–17 and 20–24 March.

where $A$ is the area of the stratum, $n_j$ is the number of whales observed on transect $j$, $e_j$ is the corresponding effort (square kilometres), and $K$ is the number of transects in that stratum. The total number of whales is the sum of the $p$'s (2). The associated variance is calculated as

$$(3) \quad \text{var}(p) = \text{var}\,[\text{mean}(p_j)].$$

The overall abundance estimate for all strata is the sum of the stratum estimates, and its variance the sum of the stratum variances.

TABLE 1. Summary of survey procedure for the four surveys. When tested against the Global Positioning System (GPS), the radar altimeter deviated less than 5 m from the GPS. 95% confidence limits are given in parentheses.

| Period | Navigation | Altimeter | Target altitude (m) | Mean speed on transects (km/h) | Transect width (m) | Observer stations |
|---|---|---|---|---|---|---|
| 15–31 March 1981 | GNS-500 Global Navigation System | Radar | 150 | 222 (217–228) | 1,400 | Right front and left rear seats |
| 10–24 March 1982 | GNS-500 Global Navigation System | Radar | 150 | 221 (218–225) | 1,400 | Right front and left rear seats |
| 9–14 April 1990 | Litton 3000 Omega | Radar and barometric | 229 | 203 (198–201) | 1,600 | Bubble windows at rear seats |
| 19–24 March 1991 | TransPak Global Positioning System | Radar, barometric, and GPS | 213 | 156 (151–160) | 1,400 | Bubble windows at rear seats |

Differences among years were tested using a bootstrapped distribution based on the combined densities in 1981, 1982, and 1991. The number of whales seen on a transect was simulated by picking at random and with replacement a density from the combined surveys and applying this density to the 1991 effort distribution. The simulations were done for each stratum separately and added up to a total abundance estimate. To generate the bootstrap distribution the procedure was repeated 1000 times. The random generator used to simulate these distributions was from Zale (1990).

Pod sighting rates (pods of whales per linear kilometre) and mean pod size estimates were compared for the observers by use of two-tailed Mann–Whitney and Kolmogorov–Smirnoff tests.

### Ice Conditions and Bathymetry of the Beluga Habitat in West Greenland

The ice conditions in the area of Davis Strait inhabited by belugas are mainly determined by the inflow of warm Atlantic water along West Greenland and by the prevailing winds. The West Greenland current creates an area of open water that usually extends north to about 67°N. However, large variations are recorded, and sometimes open water is virtually absent north of 64°N (Danish Meteorological Institute 1976, 1983–1986). In other years, open water may be present to at least Disko Island. The beluga habitat between 66°30′N and 69°30′N is an area of constantly shifting loose pack ice between the open water off West Greenland and the close pack ice in Davis Strait. The extent of the loose pack ice depends on the strength of the prevailing winds. Close pack ice is the predominant surface condition of waters in southern Baffin Bay and central Davis Strait west of 56°W.

In March 1981, little or no ice was present within 50 km of the west coast of Greenland south of 66°N. North of 66°N to Disko Island, ice was present in varying but generally small amounts along the coast (Fig. 2). The eastern part of Disko Bay was ice free, although ice covered the entrance to the bay. During 17–20 March 1981 the West Greenland coast was virtually ice free, but strong northwest winds on 20–21 March pushed the pack ice southeast with the result that by 22 March the loose ice was pushed up against the Greenland coast, effectively eliminating open water north of about 67°N for the remaining 2 d of the survey.

March 1982 differed from March 1981 by virtue of the heavy ice conditions present along the West Greenland coast (Fig. 2). With the exception of a temporary 20- to 30-km-wide shore lead, which was present from 65°N to 66°30′N on 14–17 March, there was virtually no ice-free water along the Greenland coast north of 64°N. Between 64°N and 63°N, the southern limit of the surveyed area, open water was limited to areas east of 52°30′W. In 1982, pack ice extended along the coast of West Greenland as far south as 64°N. Also, Disko Bay was completely covered with fast ice.

In April 1990, ice conditions resembled the situation in 1981 with extensive ice-free areas along the coast as far north as 68°N; there was open water in parts of Disko Bay (Fig. 2). Leads were present along the west coast of Disko Island, at the northern part of Vaigat, and north of Disko Island.

The ice conditions in March 1991 were not as severe as in 1982 but were at least as severe as in 1981 (Fig. 2). Open water was found north to 67°N, but heavy pack ice blocked Disko Bay and the area between 68°N and 69°N. North of Disko Island, no open-water areas were found.

The bathymetry of central West Greenland is characterized by shallow but highly productive banks less than 200 m deep that extend out to 50–100 km from the coast. These banks are found from Disko Island to the southern tip of Greenland. They are separated by deep channels such as the one at the southern entrance of Disko Bay which reaches depths of up to 400 m.

## Results

### Determination of Transect Width

The effective search width can be illustrated from the data collected in 1991 where the perpendicular distance to each sighting of whales was calculated. The probability of detecting a pod of whales increases with the size of the pod, and therefore, sightings rather than number of individuals were used for determining the probability of detection (Fig. 3). Some of the more distant sightings included large pods, and to reduce this bias, sightings of pods with more than 10 whales were excluded. Because of their white and luminous appearance, belugas are easily seen up to several metres below the surface, especially when they are less than 150 m from the aircraft. The probability of detection is thus higher within this zone near the trackline than it is in the more distant parts of the transect (Fig. 3). The bias is difficult to assess, as dive depth cannot be estimated from the survey platform and the whales may alter their depth in response to the passage of the platform. Narwhals are more cryptic in their coloration and thus less likely to be seen below the surface. They therefore provide a less biased estimator of the effective search width. Taking all factors into account, it seems from Fig. 3 that the probability of detection is reasonably constant for the first

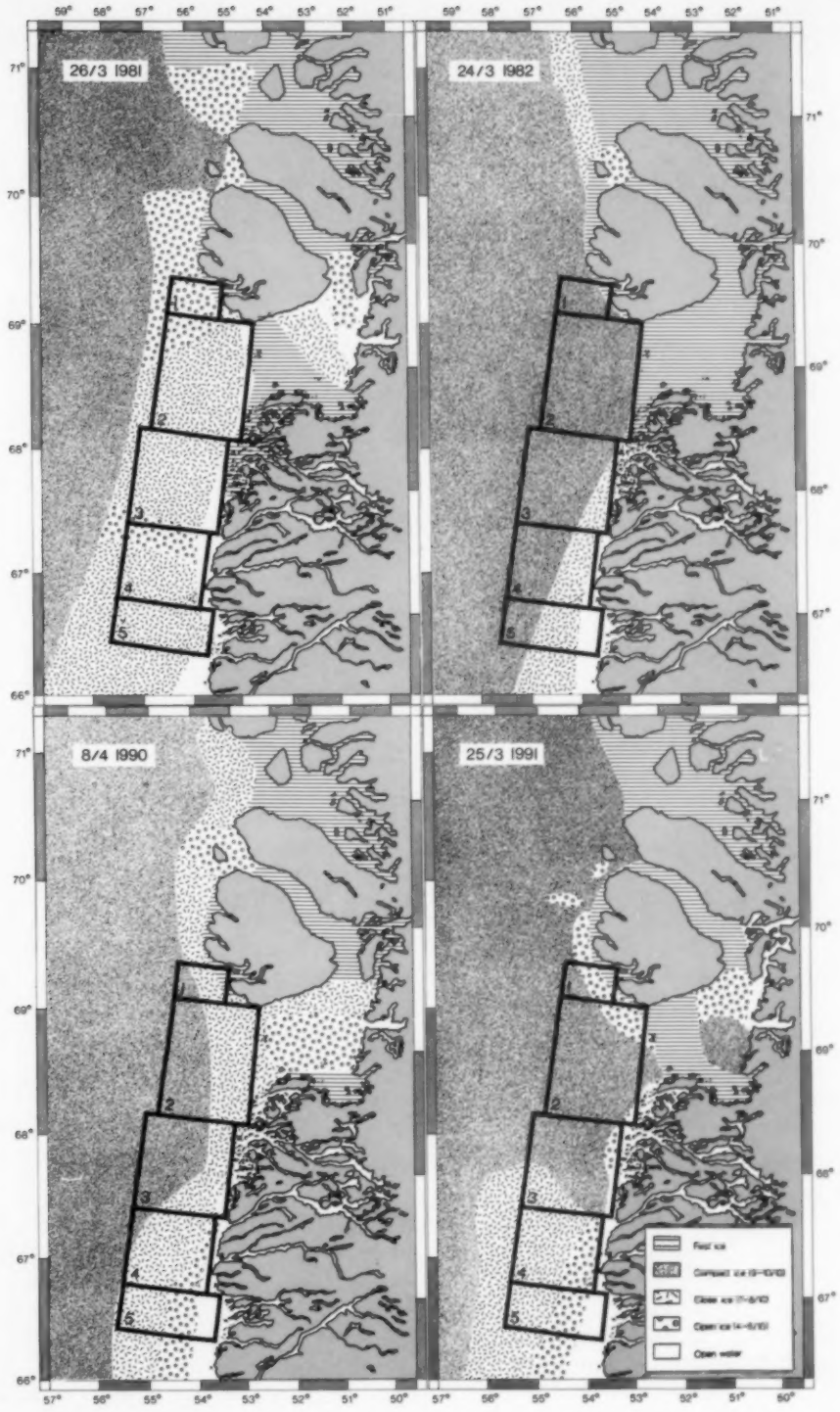



FIG. 2. Generalised ice conditions in West Greenland in 1981, 1982, 1990, and 1991 as predicted from NOAA satellite images and observations during the surveys. The rectangles show the five strata used for calculating beluga and narwhal abundance.

TABLE 2. Comparison of observer efficiency during the aerial surveys in 1981, 1982, 1990, and 1991. CV indicates coefficient of variation, n is the number of transects, and p is the probability level.

| Year | Observer | Sighting rate | *n* | CV | *p* | Mean pod size | *n* | CV | Range | *p* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1981 | 1 | 0.027 | 15 | 0.30 | | 2.74 | 34 | 0.14 | 1–11 | |
| | 2 | 0.037 | 15 | 0.21 | 0.18 | 6.22 | 46 | 0.22 | 1–50 | 0.08 |
| 1982 | 1 | 0.040 | 13 | 0.33 | | 3.28 | 82 | 0.13 | 1–24 | |
| | 2 | 0.019 | 13 | 0.30 | 0.08 | 5.96 | 45 | 0.19 | 1–35 | 0.005 |
| | 1 | 0.061 | 13 | 0.48 | | 3.28 | 82 | 0.13 | 1–24 | |
| | 3 | 0.022 | 13 | 0.36 | 0.11 | 7.03 | 40 | 0.25 | 1–60 | 0.03 |
| | 2 | 0.020 | 11 | 0.21 | | 5.96 | 45 | 0.19 | 1–35 | |
| | 3 | 0.028 | 11 | 0.37 | 0.49 | 7.03 | 40 | 0.25 | 1–60 | 0.84 |
| 1981–82 | 1 | 0.033 | 28 | 0.22 | | 3.12 | 116 | 0.10 | 1–24 | |
| | 2 | 0.029 | 28 | 0.18 | 0.51 | 6.09 | 91 | 0.15 | 1–50 | 0.001 |
| 1990 | 4 | 0.025 | 8 | 0.71 | | 2.11 | 9 | 0.31 | 1–6 | |
| | 5 | 0.039 | 8 | 0.68 | 0.19 | 1.57 | 14 | 0.14 | 1–3 | 0.88 |
| 1991 | 4 | 0.027 | 21 | 0.20 | | 3.23 | 40 | 0.13 | 1–10 | |
| | 5 | 0.020 | 21 | 0.30 | 0.32 | 2.97 | 33 | 0.30 | 1–28 | 0.08 |
| 1990–91 | 4 | 0.026 | 29 | 0.23 | | 3.02 | 49 | 0.12 | 1–10 | |
| | 5 | 0.025 | 29 | 0.33 | 0.83 | 2.55 | 47 | 0.25 | 1–28 | 0.05 |

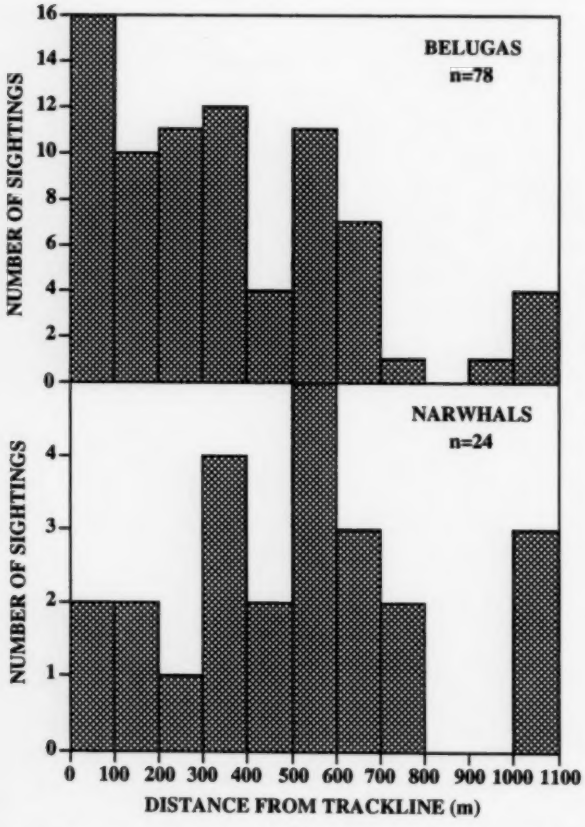



FIG. 3. Perpendicular distances to sightings of belugas and narwhals in 1991. The data are grouped by 100-m intervals. Four pods of more than 10 belugas were not included.

700 m from the trackline.

No difference could be detected between observations in the inner and the outer transects ($\chi^2 = 1.08$, df = 1, $p = 0.70$), so a transect half-width of 800 m is applied to the 1990 survey. For the following calculations, we assumed an equal probability of detection throughout a transect width of 1400 m for the surveys in 1981, 1982, and 1991.

## Observer Efficiency

Observer efficiency within the strip was tested by comparing sighting rates and mean pod size estimates for each set of observers in all four years. No significant differences could be detected for sighting rates of narwhal pods ($0.19 < p < 0.95$) or mean pod sizes ($0.06 < p < 1.00$) for any combination of observers. Sighting rates of beluga pods were also identical for all observers (Table 2), but the estimates of mean pod size showed that one observer (No. 1, Table 2) consistently made pod size estimates 50% lower than the two other observers (Nos. 2 and 3) during the 1981 and 1982 surveys. The two other observers did not differ. There was also a slight difference ($p = 0.0526$) for the mean of the pooled pod size estimates for the two observers employed in 1990 and 1991 (Table 2). The difference between the mean pod sizes for both observers in 1990–91 was 16% compared with 50% for the 1981–82 observers (Table 2). This means that the 1981–82 surveys were more heterogeneous than the later surveys, but we decided not to attempt any "corrections" of the 1981–82 survey data.

The number of sightings of pods per 50 m interval across the strip was used to test whether the observers in 1991 allocated different efforts to different regions within the strip, but no significant differences could be detected using the Kolmogorov–Smirnoff two-sample test ($p >> 0.05$). We therefore assumed an equal probability of detection for the whales appearing in the left and the right 700-m strip (800 m for 1990).

No strong correlation could be detected between distance to sighting and pod size for either narwhals (slope = –0.003, $r^2 = 0.09$) or belugas (slope = 0.006, $r^2 = 0.11$).

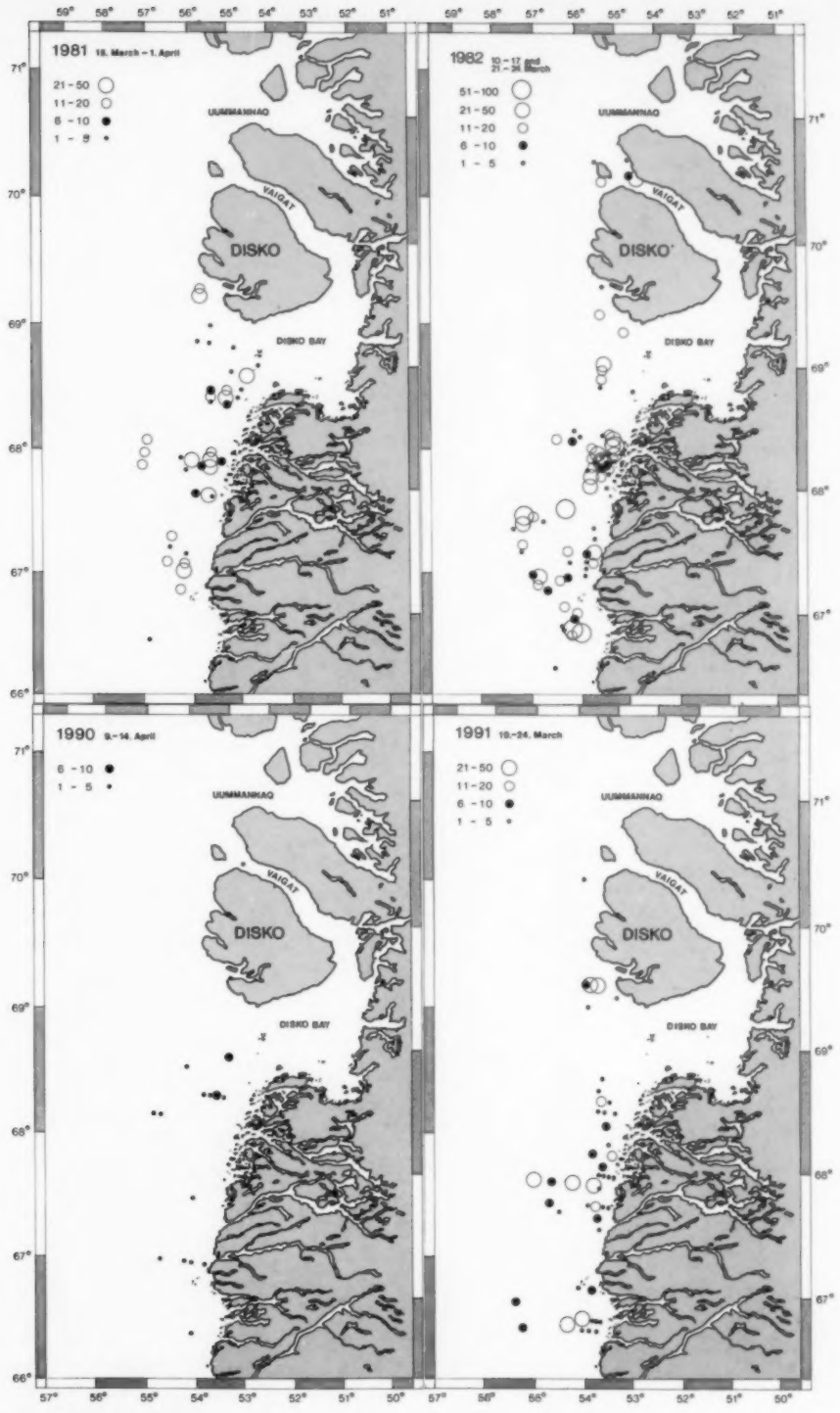



FIG. 4. Distribution of observations of belugas (including off-transect) from the aerial surveys in 1981, 1982, 1990, and 1991.

TABLE 3. Summary statistics for aerial surveys of belugas and narwhals wintering off West Greenland within the strata shown in Fig. 2. Transect segments represent 2-min intervals of each transect. 95% confidence limits are given in parentheses.

| Year | No. of transects | % with belugas | % with narwhals | No. of transect segments | % with belugas | % with narwhals | Belugas observed | Belugas/km | Narwhals observed | Narwhals/km | Coverage Stratum and area 1 1247 km$^2$ | 2 8664 km$^2$ | 3 6795 km$^2$ | 4 5185 km$^2$ | 5 3522 km$^2$ | All 25 412 km$^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1981 | 30 | 53 | 33 | 294 | 12 | 2 | 383 | 0.17 (0.10–0.33) | 30 | 0.02 (0.00–0.04) | 5.1 | 14.6 | 10.7 | 15.1 | 12.3 | 12.9 |
| 1982 | 95 | 41 | 27 | 899 | 8 | 7 | 820 | 0.13 (0.09–0.19) | 95 | 0.02 (0.01–0.03) | 22.5 | 33.5 | 37.4 | 39.9 | 32.9 | 35.3 |
| 1990 | 18 | 33 | 22 | 201 | 6 | 4 | 25 | 0.02 (0.01–0.04) | 18 | 0.02 (0.00–0.03) | 5.1 | 8.8 | 6.8 | 9.3 | 10.5 | 8.4 |
| 1991 | 36 | 50 | 22 | 490 | 7 | 3 | 234 | 0.08 (0.05–0.14) | 36 | 0.01 (0.00–0.03) | 15.5 | 15.3 | 15.4 | 16.1 | 17.5 | 15.8 |

## Distribution of Belugas

The surveys in 1981 and 1982 covered northern Davis Strait and southern Baffin Bay from 63°N to 72°N (Fig. 1). No belugas were sighted in offshore pack ice in central and western Davis Strait or in the open water along West Greenland south of 66°30′N (Fig. 4). Most belugas were sighted in the pack ice between 66°30′N and Disko Bay (69°N) (Fig. 4). In 1981, belugas were not recorded farther than 80 km of the Greenland coast, and 84% were seen within 50 km of the coast. In 1982, all belugas, with the exception of 20 (2%) seen at 55°40′N, were recorded within 45 km of the coast. This coastal area between 66°00′N and 69°30′N was surveyed during 10–17 March and again during 21–24 March 1982 (Fig. 1). Beluga distribution was similar in the two surveys with major concentrations in areas centred at about 66°45′N, 54°15′W and between 68°10′N, 54°15′W and 68°45′N, 54°30′W. Densities were higher between 68°30′N and 69°30′N during the second survey than during the first survey, but landfast ice west of Disko Island had refrozen when the area was resurveyed on 23 March 1982 and this probably prevented belugas from migrating farther north.

Disko Bay was partly ice free during the surveys in 1981, 1990, and 1991. Six transects were allocated to Disko Bay in 1982 and 1990 but only one beluga was recorded in 1990. A large plug of fast ice across the mouth of Disko Bay was present in all three years, but it was most pronounced in 1982 and 1991. This probably prevented belugas from entering Disko Bay in early March.

North of Disko Island and Vaigat, belugas were observed in a narrow recurrent lead that separated the landfast ice from the pack ice. This lead was surveyed in 1982 and 1990. In 1982, 30 and 24 belugas were observed here on 15 and 23 March, respectively, and in 1990, two belugas were seen on 14 April.

The distribution of belugas in Baffin Bay revealed by the 1981 and 1982 surveys established the framework for the surveys in 1990 and 1991. In 1990, a large area was surveyed but the survey coverage was lower (8.4%) than in previous years (Fig. 1; Table 3). Ice conditions in 1990 made searches for belugas seem futile north of 71°00′N and west of 56°00′W, and these lines delimited the surveyed area. The distribution was similar to that found in 1981 and 1982 with most belugas in the area between 67°00′N and 69°00′N and few in Disko Bay proper and in the recurrent lead at the northern opening of Vaigat (Fig. 4).

The size of the area surveyed in 1991 was reduced (Fig. 1), although the intensity of coverage was increased to 15.5% (Table 3). No search for whales was made north of 70°20′N, and Disko Bay was only checked for ice conditions from high altitude while ferrying to the airstrip. Ice conditions in 1991 were comparable with those in 1990 except that fast ice blocked the entrance of Disko Bay and probably prevented belugas from entering the bay (Fig. 2).

In 1990 and 1991, most whales were seen within 50 km of the coast. Beyond 50 km, the density declined significantly, and few belugas were seen at distances of more than 70 km from the coast (Fig. 4).

Although the belugas were widely distributed in the loose pack ice over a north-south distance of 330 km, they were not widespread. In all years, belugas were recorded on 33–53% of the transects, but they were only found in 6–12% of the 2-min transect segments (Table 3).

Most belugas were found in waters less than 100 m deep off West Greenland. They apparently also avoided the deep water (300–500 m) at the southern entrance to Disko Bay.

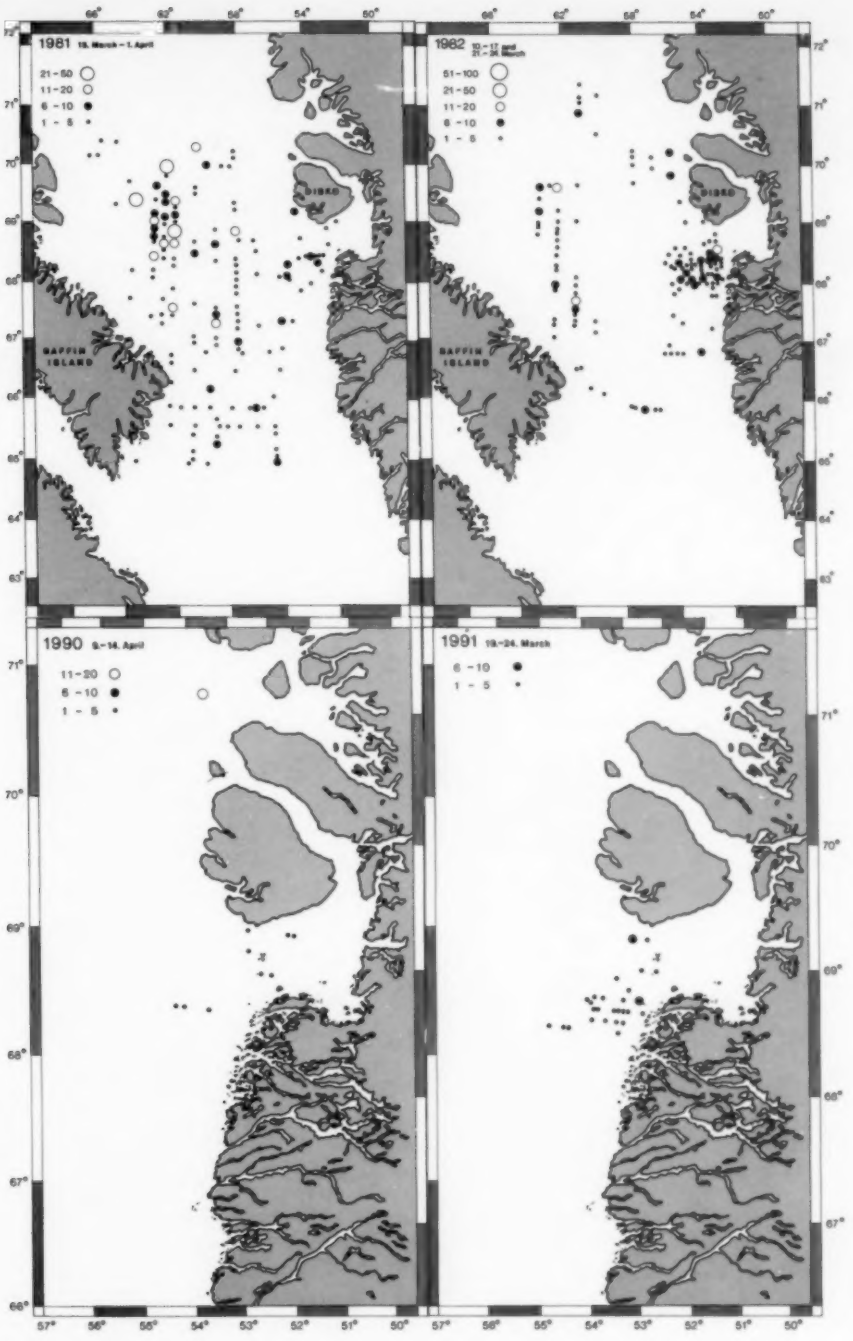



FIG. 5. Distribution of observations of narwhals (including off-transect) from the aerial surveys in 1981, 1982, 1990, and 1991.

The four years with surveys were consistent in showing that the core winter distribution of belugas in West Greenland is between 66°30′N and Disko Island (Fig. 4). In 1981 and 1982, more than 95% of the belugas were present within the area indicated in Fig. 2. This was also confirmed in 1990 and 1991 (Fig. 4), and this main core area was used for comparison of beluga abundance between years.

## Distribution and Habitat of Narwhals

Narwhals were present in both close pack ice and loose pack

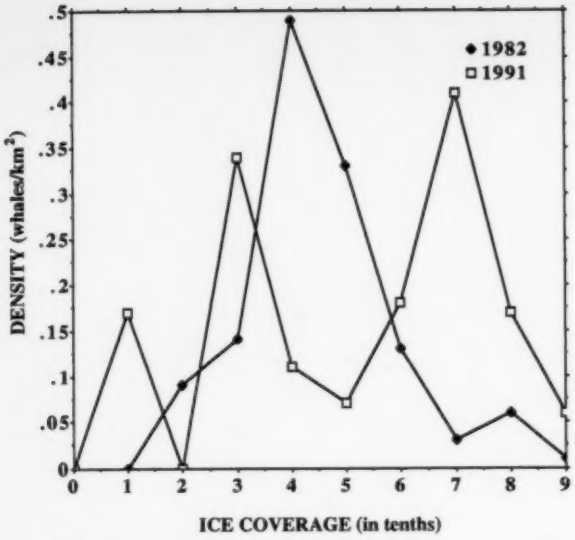



FIG. 6. Densities of belugas (per square kilometre of survey effort) for the 10 types of ice coverage in 1982 and 1991.

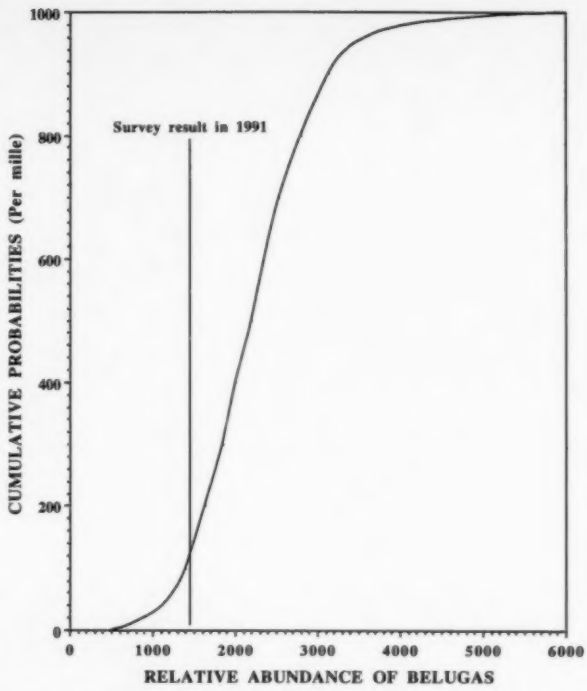



FIG. 7. Cumulative bootstrapped probability distribution of the abundances in the 1981, 1982, and 1991 surveys combined. The result from 1990 survey is indicated by the vertical line.

TABLE 4. Abundances of belugas and narwhals in five strata off West Greenland during 1981–91. Coefficients of variation are given in parentheses.

| Stratum | 1981 | 1982 | 1990 | 1991 |
|---|---|---|---|---|
| Belugas | | | | |
| 1 | 903 (1.00) | 16 (1.00) | 0 (0) | 104 (0.91) |
| 2 | 581 (0.41) | 99 (0.55) | 183 (0.58) | 189 (0.78) |
| 3 | 1633 (0.41) | 836 (0.29) | 44 (1.02) | 755 (0.37) |
| 4 | 498 (0.52) | 744 (0.28) | 54 (1.00) | 68 (0.91) |
| 5 | 0 (0) | 425 (0.59) | 10 (1.00) | 355 (0.52) |
| Overall | 3615 (0.33) | 2120 (0.19) | 291 (0.44) | 1471 (0.26) |
| Narwhals | | | | |
| 1 | 98 (1.00) | 15 (0.98) | 0 (0) | 0 (0) |
| 2 | 193 (0.47) | 281 (0.26) | 252 (0.40) | 273 (0.29) |
| 3 | 10 (0.98) | 80 (0.51) | 0 (0) | 0 (0) |
| 4 | 18 (0.98) | 31 (0.56) | 0 (0) | 0 (0) |
| 5 | 4 (0.92) | 0 (0) | 0 (0) | 0 (0) |
| Overall | 322 (0.42) | 407 (0.21) | 252 (0.40) | 273 (0.29) |

ice off the west coast of Greenland. They were widely distributed on the transects in the close pack ice west of 56°N, with observations in 1981 on 76% and in 1982 on 69% of the transects (Fig. 5). Sightings of animals at 70°40′N, the northern limit of the survey area, in both 1981 and 1982 suggest that narwhals are also present farther north in Baffin Bay in late March.

In the loose pack ice along West Greenland, most narwhals were found at the southern entrance to the Disko Bay where deep water prevails (Fig. 5). No narwhals were recorded in open pack ice or in ice-free waters along West Greenland south of 66°30′N.

Narwhals were found exclusively in waters with greater than 5/10 ice cover and most sightings were made in 9/10 ice cover.

There were large changes in the number of sightings of narwhals along West Greenland in 1982. During the first survey (10–17 March), a total of 139 narwhals were seen on transect, but with a comparable effort during the last survey (21–24 March), only 31 were seen on transect, which was similar to the 1981 survey (17–24 March). It is likely that the narwhals had moved out of the area between the surveys. During the first 1982 survey, 66% of the transect segments flown had 9/10 ice cover, the narwhals' preferred habitat. During the second 1982 survey, the comparable figure was 50%; during the 1981 survey, this figure was 44%.

## Ice Conditions and Beluga Habitats

In all years, a rather limited proportion (2–7%) of the surveyed area had less than 4/10 ice cover, and large open-water areas were only surveyed in 1981 and 1990. In all years the most intensively surveyed ice habitat was between 9/10 and 10/10 ice cover. This habitat had the highest survey intensity in 1982, a year of heavy ice conditions. The ice types surveyed in 1982 were significantly different from the types surveyed in 1990 and 1991 (Kolmogorov–Smirnov, $p < 0.05$) whereas 1990 and 1991 were not significantly different ($p > 0.05$). The observations of ice coverage in 1981 were not collected in tenths and hence the difference between years could not be tested for 1981.

The highest observed densities of belugas were in the pack ice with between 4/10 and 8/10 ice cover (Fig. 6). Few belugas were seen in open water or in cracks in the close pack ice. On 23 March 1991, herds of belugas were seen moving northward in the shore lead along the west coast of Disko Island. They obviously used the loosening of the pack ice for penetrating to the north, but the passage was blocked by landfast ice north of Disko Island (Fig. 2). The distribution of beluga densities on the various ice

TABLE 5. Pod sizes of belugas recorded during aerial surveys off West Greenland, excluding belugas seen off-transect, in March–April 1981–82 and 1990–91

| | 1981 (%) | 1982 (%) | 1990 (%) | 1991 (%) |
|---|---|---|---|---|
| 1 | 30 | 32 | 66 | 51 |
| 2 | 26 | 19 | 13 | 12 |
| 3 | 8 | 12 | 13 | 8 |
| 4 | 11 | 7 | 0 | 8 |
| 5 | 4 | 8 | 4 | 7 |
| 6 | 4 | 4 | 4 | 4 |
| 7 | 3 | 1 | 0 | 0 |
| 8 | 1 | 4 | 0 | 3 |
| 9 | 0 | 2 | 0 | 0 |
| +10 | 13 | 11 | 0 | 7 |
| *n* | 74 | 167 | 23 | 73 |

coverages showed obvious differences between 1982 and 1991, but the two distributions were not statistically different (Wilcoxon, $p > 0.05$). The large difference in group sizes between the 1981–82 and 1991 surveys (see below) prevented more detailed analyses of the effect of year on the clumping of belugas in different habitats.

### Estimates of the Relative Abundance of Belugas and Narwhals along West Greenland

The modified ratio method for estimating variances from transects of equal lengths produced variance estimates almost identical to those developed for systematic surveys with correlated densities. Both the ratio methods for variance calculations of transects of equal and unequal lengths were sensitive to small changes in effort and sighting rates and were therefore considered less reliable than the bootstrap variance determination. Although the bootstrap variance estimates were slightly higher (CV's 8–9% larger) than the analytical variance estimates, we considered them superior because of their relative insensitivity to the data set; hence, only bootstrapped variance estimates are presented here (Table 4).

Belugas were present on 33–53% of the transects but only on 6–12% of the 2-min segments in all four surveys (Table 3). The mean number of belugas observed on transect per linear kilometre declined from 0.142 (95% CI: 0.099–0.192) in 1981–82 to 0.060 (95% CI: 0.035–0.099) in 1990–91 (Table 3).

For the comparison of trends in abundance the survey in 1990 was excluded because of its later timing. The combined bootstrapped cumulative probability distribution of the 1981, 1982, and 1991 surveys shows that the probability of achieving the abundance estimate obtained in 1991 or less is about 13%, under the assumption that all three surveys provided random samples of the same population (Fig. 7).

Narwhals were present on 22–33% of the transects but only on 2–7% of the 2-min segments (Table 3). Narwhals were generally less abundant than the belugas within the strata in West Greenland. They were primarily found in the northern strata but they seemed to be more dispersed in 1981 and 1982 (Fig. 5; Table 4). Both the number of narwhals sighted per linear kilometre and the overall narwhal abundance did not differ significantly between years (Tables 3 and 4).

TABLE 6. Age classification of belugas recorded during aerial surveys off West Greenland in March–April 1981–82 and 1990–91.

| | 1981 | 1982 | 1990 | 1991 | Photo from 1991 |
|---|---|---|---|---|---|
| White | 84% | 66% | 61% | 62% | 50% |
| Gray | 16% | 32% | 35% | 35% | 42% |
| Newborns | 0% | 2% | 4% | 3% | 8% |
| Mother/calf | — | — | 3 | 11 | 5 |
| Total *n* | 189 | 383 | 28 | 92 | 26 |

### Pod Characteristics and Age Classification of Belugas and Narwhals

Pod sizes ranged from 1 to 60, but values given for belugas in pods larger than 10 were frequently estimates rather than counts (Table 5). The mean of pod size estimates from the surveys in 1981–82 ($\bar{x} = 4.9$, CV = 0.10) was significantly larger than the mean of pod size estimates from the surveys in 1990–91 ($\bar{x} = 2.8$, CV = 0.13). The mean pod size was 1.8 (CV = 0.16) in 1990 and 3.1 (CV = 0.15) in 1991. Pod size distributions in both 1990 and 1991 differed significantly with respect to location and dispersion (Kolmogorov–Smirnoff, $p < 0.05$) from those in 1981 and 1982 and from each other (Table 5). The differences between the 1991 survey and 1981 and 1982 surveys are specifically seen in the number of single animals; there were many more sightings with a pod size of one in 1991 than 1981–82. Also, the number of large pods (greater than nine) was greater in 1981–82 than in 1991 (Table 5).

Approximately two thirds of the belugas that could be assigned to an age class were white adults (Table 6). Between 0 and 3% were tentatively classified as newborns but this observation may be disputable, as the presence of newborns is difficult to assess from an aircraft. An estimate of 8% newborns in a group of 26 animals was made from a photograph taken in 1991 (Table 6), suggesting that it may be more efficient to assess pod composition with photographs than from visual observations alone.

Belugas accompanied by dark or greyish calves were seen once in 1990 (14 April) and eight times during 19–23 March 1991. On three occasions in 1991, the observed calves were judged to be newborn on the basis of their length relative to the lengths of accompanying adults.

There was no statistically significant difference ($p = 0.63$) in mean pod size of narwhals in the strata along West Greenland between 1981–82 ($\bar{x} = 2.2$, CV = 0.09) and 1990–91 ($\bar{x} = 2.3$, CV = 0.12).

## Discussion

The surveys conducted in 1981 and 1982 over close pack ice in central Baffin Bay and along the east coast of Baffin Island resulted in no observations of belugas. The tendency of belugas to remain in the loose pack ice near the Greenland coast was confirmed in all surveys. Most of the animals sighted were within 50 km of shore and few were seen up to 75 km from the coast. Furthermore, belugas were not found along the fast ice edge in Vaigat and Uummannaq during any of the surveys. Their present shallow-water (<200 m), nearshore winter distribution is consistent with the winter distribution of belugas in southwest

Greenland earlier this century (Degerbøl and Nielsen 1930; Heide-Jørgensen 1994) and with their summer distribution in other areas (Kleinenberg et al. 1969; Stewart and Stewart 1989).

The surveys in 1981 and 1982 found no belugas along the West Greenland coast between 63°N and 66°30′N. Nor were any observed during an aerial survey off West Greenland between 65°N and 67°N in March 1978 (MacLaren Marex 1979). Their absence in this region in March 1981 was attributed to the complete absence of ice along the coast in that year. However, in 1982, apparently suitable habitat (5/10 to 8/10 ice cover) was present along the coast to 64°N and similar habitat was also encountered far offshore during surveys from 63°N to 64°N. Similar ice conditions were present south to 66°N in March 1978 (MacLaren Marex 1979).

The surveys in 1981 and 1982 demonstrated that the major wintering area for narwhals is the close pack ice of northern Davis Strait and southern Baffin Bay. They are widely distributed in the pack ice of 9/10 coverage and they extend north to at least 72°N in March. Narwhals are most likely distributed throughout northern Baffin Bay including the North Water. Along West Greenland, only the mouth of Disko Bay attracts significant numbers of narwhals. This area has much greater water depths than elsewhere along West Greenland. Their wide distribution and preferred habitat of heavy pack ice make it difficult to monitor narwhal abundance from surveys in West Greenland.

The survey procedures varied slightly among years, but most changes would have improved the probability of detecting whales and reduced the downward biases in the 1990–91 surveys compared with those in 1981–82. The surveys in 1981 and 1982 were conducted using a lower flight altitude (152 m) than that used in the 1990–91 surveys, but the effect of this difference cannot be assessed. The lower ground speed used in 1990 and 1991 should have generally improved the probabilities of detecting animals. In 1991, ground speed was significantly lower than in any of the other years, altitude was slightly lower than in 1990, and the more conservative transect width of 700 m was used. The aircraft used in 1990 and 1991 was equipped with bubble windows at each rear seat, making it well suited for marine mammal surveys. The transect width was not reduced by a blind swath straight below the aircraft, as it was for the Twin Otter aircraft used in 1981 and 1982. In addition the observers used during the surveys in 1990 and 1991 showed a more homogeneous efficiency than the observers used in 1981 and 1982. The problem of defining the optimum transect width is of minor or no importance when using aerial surveys for indexing the relative abundance of whales as long as survey procedures and treatment of data are consistent between surveys.

The similarity in the distribution of sightings in all four years indicates that there was no major change in the distribution of wintering belugas. We conclude that the area of approximately 25 000 km$^2$ used for the extrapolations demarcates the main wintering ground. The area, as constructed, is probably large enough to allow for small-scale year-to-year changes in distribution.

The differences between the results of the 1990 survey and those of the other three surveys exceed what may be explained only by differences in survey procedures. The survey procedures used in 1990 resembled those used in 1981–82, and if anything, the procedures used in 1990 were probably better for detecting belugas than those used in 1981–82. The later timing of the survey in 1990 may have violated some of the assumptions for comparing surveys. Ice conditions in April 1990 when the survey was conducted were severe, although immediately prior to the survey, a mild and windy period had changed the ice coverage. During and after the survey, close pack ice was present north of 71°N, close to the west coast of Disko Island and throughout northern Baffin Bay. No belugas were observed along the west coast of Disko Island or north of Disko to 71°20′N except for a few at the opening of Vaigat where currents usually ensure that there is open water throughout the winter. We believe that belugas use the open-water passage west of Disko for their northward spring migration, but no signs of such a movement were evident during the aerial survey in 1990, and ice conditions north of 71°N were probably not suitable for belugas. However, neither the timing nor the route of beluga migration in Baffin Bay is well known. It is possible that a smaller proportion of the belugas that winter in Baffin Bay was present off West Greenland in 1990 than in the other years. A large ice entrapment of belugas occurred in Disko Bay in early February 1990, where an estimated 400 belugas were taken by hunters (Siegstad and Heide-Jørgensen 1994). This mortality, plus the whales that were killed but lost and others that may have died at adjacent but undiscovered entrapments, could have severely reduced the density of belugas in the following months. Whether the low abundance observed in 1990 can be explained by the exceptionally high mortality earlier in the same winter remains an open question.

Belugas are not found in open water at their wintering grounds, but rather in the loose and rapidly shifting pack ice or in leads or cracks in the close pack ice. The amount of suitable habitat varies between years and may affect beluga distribution such that increased ice coverage increases the clumping of whales in leads and cracks and increases the number of transects with no belugas. It may also alter the size of the beluga pods, leading to their concentration in large groups. The first two effects can be seen when comparing results from 1981, when open water was maximal, with those from 1982, when the most severe ice conditions in any of the surveys were documented. The suggested effect of increased pod size of ice coverage characteristics cannot be confirmed, as the smallest pod sizes were found in 1990 and 1991, when ice coverage was intermediate between that in 1981 and 1982. Clumping and transects without whales may decrease the density estimate in a systematic survey and will certainly increase the variance. For the comparison between years, it is, however, important to note that even though the relationship between relative abundance and ice conditions cannot be fully elucidated, it may still not confound the detected trends. The ice conditions in 1981 and 1982 represent the extremes among the four years with surveys, but these two years, involving identical survey procedures, revealed similar densities of belugas. There are reasonable grounds for excluding the 1990 survey due to its later timing whereas the timing of 1991 survey was similar to that in 1981 and 1982, with improved survey techniques and ice conditions intermediate between 1981 and 1982. The 1991 survey, nevertheless, provided a lower relative abundance of belugas wintering off West Greenland compared with the 1981–82 surveys. This is evident from both the number of whales observed per linear kilometre and the estimated abundance in the entire area.

The 1991 estimate of abundance for belugas is low compared with the 1981 and 1982 surveys, but the probability ($p$ = 13%) of obtaining the observed values, or even lower values in 1991, is not low enough to support a statistically unambiguous conclusion. The alternative hypothesis, that the 1991 survey was actually sampling a population that was only 75% as large as in

1981–82, can, using the observed density distribution for 1981–82 and the effort distribution of 1991, be examined using a bootstrap. The probability of observing an abundance estimate significantly lower than the 1981–82 estimate is 15% if a one-sided bootstrap applying a 5% significance level is used. If the abundance in 1991 was actually half that of the 1981–82 level the probability of detecting this change would have increased to 54%. Neither the analysis of Type I error nor that of Type II error is conclusive. However, all indications point to the conclusion that the number of belugas in West Greenland waters has declined since the early 1980's.

Narwhals are widely distributed in the Baffin Bay and Davis Strait pack ice during winter months (McLaren and Davis 1983). It is therefore interesting to note that the surveys produced relatively constant estimates of narwhals abundances although narwhals are not restricted to the strata defined for the winter distribution of belugas. Also, the same series of surveys gave similar estimates of walrus abundance in West Greenland (Born et al. 1994).

The improved coverage and survey technique in 1991, with an actual ground speed only 70% that of the 1981 and 1982 surveys, should have ensured a higher probability of detecting whales than in any of the previous surveys. Both the 1990 and 1991 surveys provide evidence for a much reduced wintering population of belugas off West Greenland. This apparent decline should at the very least prompt more careful monitoring of the stock and draw attention to the likely causes of the decline.

## Acknowledgements

We thank our pilots G. Drysdale, T. MacDonald, L. Webber, and D. Louden of Petro-Canada, O. Kesler of Kesler Avaiation, and L. Petersen of Danish Air Survey for excellent skills and cooperation during the operations. P.L. McLaren, S.R. Johnson, and G.W. Miller conducted the surveys in 1981 and 1982. E.W. Born, F. Larsen, R. Reeves, and T. Smith commented on an earlier version of this paper. This study was funded by the Arctic Pilot Project, Greenland Fisheries Research Institute, and the Aage V. Jensen Charity Foundation.

## References

Born, E.W. 1986. Observations of narwhals (*Monodon monoceros*) in the Thule area (NW Greenland), August 1984. Rep. Int. Whaling Comm. 36: 387–392.

Born, E.W., M.P. Heide-Jørgensen, and R.A. Davis. 1994. The walrus in West Greenland. Meddr. Grønland, Biosci.(In press)

Caughley, G. 1977. Sampling in aerial survey. J. Wildl. Manage. 41: 605–615.

Caughley, G., and G.C. Grigg. 1981. Surveys of the distribution and density of kangaroos in the pastoral zone of South Australia, and their bearing on the feasibility of aerial survey in large and remote areas. Aust. Wildl. Res. 8: 1–11.

Cochran, W.G. 1977. Sampling techniques. John Wiley & Sons, New York, NY. 428 p.

Danish Meteorological Institute. 1976. West ice investigations. Part 1. West ice histograms 1959–1975. Danish Meteorological Institute, Nautical Department, Copenhagen, Denmark.

Danish Meteorological Institute. 1983–86. Isforholdene i de grønlandske farvande (The ice conditions in the Greenland waters). Publikationer fra Det Danske Meteorologiske Institut Årbøger. København. Danish Meteorological Institute, Nautical Department, Copenhagen, Denmark.

Degerbøl, M., and N.L. Nielsen. 1930. Biologiske iagttagelser over og maalinger af hvidhvalen (*Delphinapterus leucas* (Pall.)) og dens fostre (with an English summary). Meddr. Grønland. 77(3): 119–144.

Finley, K.J., and W.E. Renaud. 1980. Marine mammals inhabiting the Baffin Bay North Water in winter. Arctic 33: 724–738.

Freeman, M.M.R. 1968. Winter observations on beluga (*Delphinapterus leucas*) in Jones Sound, NWT Can. Field-Nat. 82: 276–286.

Heide-Jørgensen, M.P. 1994. Distribution, exploitation and population status of white whales (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*) in West Greenland. Meddr. Grønland, Biosci. (In press)

Kapel, F.O. 1977. Catch of belugas, narwhals, and harbour porpoises in Greenland, 1954–75, by year, month and region. Rep. Int. Whaling Comm. 27: 507–520.

Kingsley, M.C.S., I. Stirling, and W. Calvert. 1985. The distribution and abundance of seals in the Canadian High Arctic, 1980–82. Can. J. Fish. Aquat. Sci. 42: 1189–1210.

Kleinenberg, S.E., A.V. Yablokov, B.M. Bel'kovich, and M.N. Tarasevich. 1969 Beluga (*Delphinapterus leucas*): investigation of the species. Moscow. Israel Program for Scientific Translations, Jerusalem. vi + 376 p.

MacLaren Marex. 1979. Report on aerial surveys of marine mammals & birds in southern Davis Strait & eastern Hudson Strait in March 1978. Rep. for Esso Resources Canada Ltd., Arctic Petroleum Operators Association. Project No. 146.

McLaren, P.L., and R.A. Davis. 1983. Distribution of wintering marine mammals off West Greenland and in southern Baffin Bay and northern Davis Strait, March 1982. Rep. by LGL Ltd., Toronto, for Arctic Pilot Project, Calgary, Alta. 98 p.

Reeves, R.R., and E. Mitchell. 1987. Distribution and migration, exploitation, and former abundance of white whales (*Delphinapterus leucas*) in Baffin Bay and adjacent waters. Can. Spec. Publ. Fish. Aquat. Sci. 99: 34 p.

Sergeant, D.E., and P.F. Brodie. 1975. Identity, abundance, and present status of populations of white whales, *Delphinapterus leucas*, in North America. J. Fish. Res. Board. Can. 32: 1047–1054.

Siegstad, H., and M.P. Heide-Jørgensen. 1994. Ice entrapments of narwhals (*Monodon monoceros*) and white whales (*Delphinapterus leucas*) in Greenland. Meddr. Grønland, Biosci. (In press)

Smith, T.G., M.O. Hammill, D.J. Burrage, and G.A. Sleno. 1985. Distribution and abundance of belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, in the Canadian High Arctic. Can. J. Fish. Aquat. Sci. 42: 676–684.

Stewart, B.E., and R.E.A. Stewart. 1989. *Delphinapterus leucas*. Mamm. species 336: 1–8.

Strong, J.T. 1988. Status of the narwhal, *Monodon monoceros*, in Canada. Can. Field-Nat. 102: 391–398.

Vibe, C. 1950. The marine mammals and the marine fauna in the Thule District (northwest Greenland) with observations on ice conditions in 1939–41. Meddr. Grønland. 150(6): 1–117.

Zale, R.S. 1990. Power basic. Borland International, Inc., Spectra Publishing, Sunnyvale, Calif. 354 p.

# A Bioenergetics Analysis of Diel Vertical Migration by Kokanee Salmon, *Oncorhynchus nerka*

Mark S. Bevelhimer[1]

*Graduate Program in Ecology, The University of Tennessee, Knoxville, TN 37996, USA*

and S. Marshall Adams

*Environmental Sciences Division, Oak Ridge National Laboratory,[2] Oak Ridge, TN 37831, USA*

Bevelhimer, M.S., and S.M. Adams. 1993. A bioenergetics analysis of diel vertical migration by kokanee salmon, *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 50: 2336–2349.

Diel vertical migration of fishes is probably a result of the combined effects of several selective forces, including predator avoidance, foraging efficiency, and bioenergetic efficiency. We considered both foraging efficiency and energetic efficiency as a combined effect which we called growth maximization. The importance of growth maximization as a selective force was evaluated with a bioenergetics-based model to estimate growth rates of various migration scenarios of kokanee salmon, *Oncorhynchus nerka*. Environmental parameters (temperature and zooplankton distributions) in the model were obtained from a North Carolina reservoir with an established population of kokanee. The simulations demonstrated that vertical migrations can be energetically advantageous when kokanee and their prey are thermally segregated and that ontogenetic and seasonal differences in the optimal migration strategy should be expected. The general rule for vertical migration as determined from the simulations is to feed where net energy intake is maximized and then reside when not feeding where energetic costs are minimized and food is digested to the point that consumption during the next feeding period is not limited by the amount of undigested food remaining in the stomach. Data obtained from vertical gill nets and hydroacoustics were compared with model predictions.

La migration verticale nycthémérale des poissons résulte probablement des effets combinés de plusieurs forces, notamment l'évitement des prédateurs, l'efficacité de la recherche de nourriture et l'efficacité bioénergétique. Nous considérons l'efficacité de la recherche de nourriture et l'efficacité énergétique comme un effet combiné que nous appelons maximisation de la croissance. On a évalué l'importance de cet effet comme force de sélection à l'aide d'un modèle fondé sur la bioénergie afin d'estimer le taux de croissance dans le cadre de différents scénarios de migration du kokani, *Oncorhynchus nerka*. Les paramètres environnementaux (température et répartitions du zooplancton) du modèle provenaient d'un réservoir de la Caroline du Nord où s'est établie une population de kokani. Les simulations ont montré que des migrations verticales peuvent être intéressantes sur le plan énergétique lorsque le kokani et ses proies ne vivent pas dans les mêmes zones thermiques et qu'on devrait s'attendre à des différences ontogénétiques et saisonnières dans la stratégie de migration optimale. La règle générale d'une migration verticale établie à partir des simulations consiste à s'alimenter lorsqu'il y a maximisation de l'apport énergétique net puis, en période de non alimentation, à se tenir à un endroit où les dépenses énergétiques sont réduites au minimum et que la digestion des aliments est telle que la consommation pendant la période d'alimentation suivante n'est pas limitée par la quantité d'aliment non digéré demeurant dans l'estomac. Les données obtenues par les filets maillants verticaux et des données hydroacoustiques ont été comparées aux prévisions du modèle.

*Received November 27, 1990*
*Accepted May 31, 1993*
*(JA824)*

*Reçu le 27 novembre 1990*
*Accepté le 31 mai 1993*

The various hypotheses proposed to explain the adaptive significance of diel vertical migration of fish can for the most part be grouped into two categories: (1) those relating to maximization of growth (i.e., net energy assimilation) and (2) those relating to the minimization of the probability of premature mortality. The first category includes (i) maximizing food intake rate, either by feeding where prey densities are highest (Janssen and Brandt 1980; Munk et al. 1989) or by stimulating digestion via temperature selection (Wurtsbaugh and Neverman 1988), and (ii) maximizing assimilation efficiency by minimizing energetic costs (Brett 1971a). The second category includes (i) spatial and/or temporal avoidance of predators (Eggers 1978; Clark and Levy 1988) and (ii) avoidance of prolonged exposure to potentially stressful environmental conditions (e.g., high temperature or low dissolved oxygen (DO); Neill and Magnuson 1974; Olla et al. 1985). These selective forces are not independent of each other, and given the expected seasonal and ontogenetic variation in the relative importance of these forces, it is unreasonable to expect that any one can adequately explain the complex migration patterns of even a single species. Levy (1990a) suggested that vertical migration by juvenile sockeye salmon, *Oncorhynchus nerka*, may be a three-way compromise between foraging, predator

[1]Present address: Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6038, USA.

[2]Managed by Martin Marietta Energy Systems, Inc., under contract No. DE-AC05-840R21400 with the U.S. Department of Energy.

avoidance, and metabolic efficiency. Although it is difficult to evaluate the adaptive value of these selective forces concurrently because of the difficulty in defining a common currency (McNamara and Houston 1986), evaluation of one of these individually can contribute toward understanding this rather complex phenomena.

Studies mentioned above evaluated individual components of growth such as energetic cost minimization, assimilation efficiency, or food consumption rate, but we chose to consider the cumulative response, which is growth itself. The growth maximization hypothesis as discussed here is actually just a broadened definition of Brett's (1971a) bioenergetics hypothesis and proposes that vertical migration through a range of temperatures can serve to maximize net energy assimilation by a combination of maximizing food consumption and minimizing metabolic costs. According to this hypothesis, the environmental conditions that would favor such movements are a spatial separation between optimal food densities and optimal thermal habitat. Under such conditions, a fish could reside at preferred temperatures where food is scarce (thus minimizing metabolic costs), reside where food is abundant but temperature is physiologically unfavorable (thus maximizing food consumption), reside where neither condition is optimal, or move between two (or more) depths taking advantage of the benefits of both (all).

Our primary objective was to evaluate the theoretical validity of the growth maximization hypothesis. To do this, we developed a detailed bioenergetics model which simulates the food consumption and growth of kokanee salmon (the nonanadromous freshwater variety of *O. nerka*) under various vertical migration scenarios. This analysis is similar to that performed by Swift (1976) on the vertical migration of *Chaoborus* larvae. We attempted to evaluate predictions of the model against the results of gillnet sampling and hydroacoustic surveys.

Input parameters for the model (temperature profiles and zooplankton densities and distributions) were derived from data collected from Nantahala Lake (western North Carolina) from 1985 to 1988. Nantahala Lake has an established population of kokanee and a seasonal spatial heterogeneity of temperature and food. It is characterized by a wide range of well-oxygenated thermal habitats with summer surface temperatures exceeding those normally experienced by this fish in the Pacific northwest. In summer, large zooplankton, the primary food of adult kokanee (Northcote and Lorz 1966; Schneidervin and Hubert 1987), are concentrated in the upper portion of the water column where temperatures are typically greater than 15°C, the physiological optimum of this species (Brett 1971a).

Diel vertical migration of juvenile sockeye salmon (the anadromous variety of this species) has been documented and extensively studied by several investigators (Narver 1970; Brett 1971a; Eggers 1978; Levy 1987, 1990a, 1990b). The vertical migration of adult sockeye at sea is not as well documented, although Pearcy et al. (1984) reported increased catches of adults at the surface at night. Northcote et al. (1964) observed vertical migration in adult kokanee, but little has been reported on juvenile kokanee. The general migration pattern observed for this species is an ascent to near the surface at dusk, followed by a partial descent during the night, a second ascent to the surface before dawn, and then a return to depths of 30–50 m where they reside during the day. Exceptions to this general pattern were reported by Northcote et al. (1964) and Levy (1990b).

In order to focus on growth maximization as a contributing factor to vertical migration, we attempted to minimize the influence of predator avoidance in this study by developing the model for adult kokanee (>300 mm), whose risk of predation in Nantahala Lake is negligible. It is unlikely that the large piscivores in this system (largemouth bass, *Micropterus salmoides*, walleye, *Stizostedion vitreum*, and channel catfish, *Ictalurus punctatus*) would regularly consume fish larger than 250 mm. To minimize the influence of migratory behavior related to spawning, which occurs around the second week of October in this population, most of the sampling was performed from May to August.

## Methods

### Lake Description

Nantahala Lake (Macon County, North Carolina), located in the southern Appalachian Mountains at an elevation of 918 m, was impounded in 1942 principally for hydroelectric generation. Total surface area is 6.5 $km^2$, maximum depth is 69 m, and average depth is 26 m. Nantahala Lake is mesooligotrophic and has steep rocky or sandy sides with a relatively small littoral zone. Because of regular water withdrawal for hydroelectric operation, annual drawdown of 10–15 m by late fall is normal. In years of low rainfall, drawdown can exceed 35 m.

Kokanee were stocked in Nantahala Lake for 2 or 3 yr in the early 1960s, and the population is presently self-supporting. Fall spawning migrations occur in the relatively pristine Nantahala River and its tributaries.

### Field Data Collection

Temperature and DO profiles were determined with a Hydrolab Surveyor II at least once during each sampling trip. Secchi depths were also determined, and light penetration (PAR) was quantified with a photometer (Li-Cor model LI-188B) on a few occasions.

Zooplankton were collected by 1- to 2-min horizontal tows with a Clarke-Bumpus zooplankton sampler (10-cm diameter, 153-μm mesh) at five to seven depths between 3 and 27 m. These samples were collected in the same area where kokanee were captured. Samples were normally collected during midday (10:00–15:00) and on one occasion at night. Samples were preserved in 10% formalin for later identification and enumeration. Zooplankton larger than 1 mm (the size most likely to be consumed by adult kokanee as determined from stomach samples) were counted and identified to genus.

Six vertical gill nets (4.6 m wide × 30.5 m long; Horak and Tanner 1964) were used to collect kokanee and determine their depth distribution on 10 occasions (26 total nights) from May 1985 to June 1988. A different mesh size was used on each net (1.9, 2.5, 3.2, 3.8, 4.4, and 5.1 cm square measure). Nets were usually placed where maximum depths were 27–30 m so that the nets fished the entire water column. Because daytime sets proved mostly ineffective for all species, nets were set overnight, usually in two groups of three. Fish were removed from the nets early in the morning while still fresh and often alive, and the depth of capture was noted. Kokanee stomachs were pumped (Seaburg 1957) and the contents preserved in 10% formalin. The fish were then tagged for later identification and preserved on dry ice. Although the adult kokanee were of most interest, all kokanee captured in the nets were collected and processed. Depth of capture was usually noted for nontarget species as well. Upon return to the laboratory, the kokanee were weighed, measured,

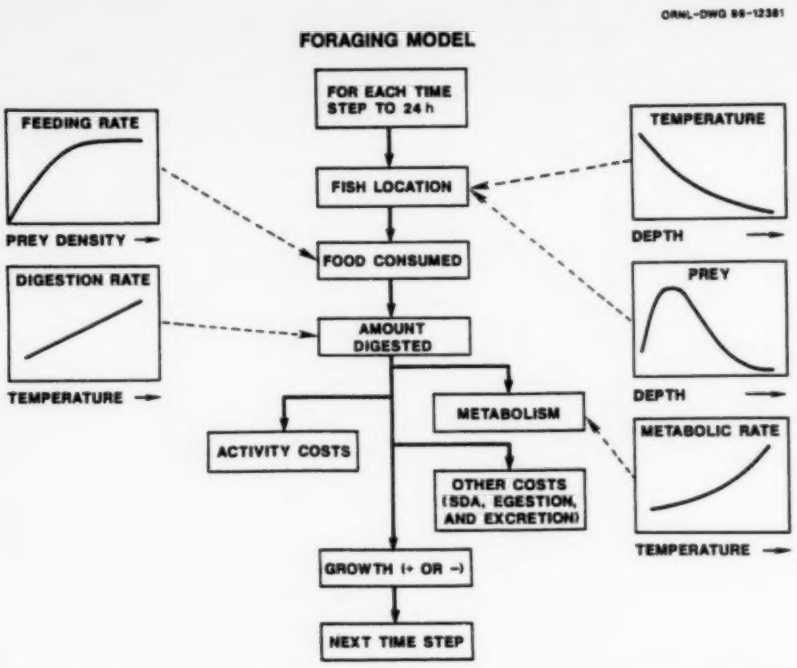



FIG. 1. Flow chart of bioenergetics model used to predict growth resulting from various vertical migration strategies of kokanee salmon.

and sexed.

Hydroacoustic surveys were conducted on 28–29 July 1987 and 7–8 June 1988 to estimate the distribution and vertical migration patterns of kokanee. A dual-beam/echo integration system was used for acoustic data acquisition and processing (Thorne 1983). This system consisted of the following equipment: 120-kHz, $10 \times 25°$ BioSonics dual-beam transducer installed in a towed body, echo sounder with dual receivers, digital echo integrator, dual-beam processor, thermal chart recorder, microcomputer, and videocasette recorder.

Preliminary surveys were performed to determine the general location of highest kokanee concentration. Once an area with sufficient densities was located, 30-min surveys (six in 1987 and nine in 1988) were then conducted during a 24-h period. Each survey consisted of shore-to-shore transects in a zigzag pattern. The 1987 and 1988 surveys were in two different areas of the lake.

Kokanee depth distribution and target strength analyses were performed from recorded data. Target strengths (decibels) from each marked fish were roughly translated to fish length (millimetres) with Love's (1971) equation. Simultaneous gillnet data were also used to estimate the size of kokanee being tracked by hydroacoustics. The data were analyzed in 2-m depth intervals beginning at 2 m. Because the volume of water being sampled by this technique is conical, a correction was applied to the data so that the estimate of the number of targets marked in each depth interval represented an equal volume of water sampled.

## Bioenergetics Model

### *Basic model operation*

The bioenergetics model is based on a mass balance equation which accounts for energy from consumed food as it is partitioned among various energetic costs and the growth of an organism. Although the model is most commonly used to estimate food consumption given estimates of growth and energetic costs (Kitchell et al. 1977; Stewart et al. 1981), it can also be used to estimate growth given estimates of food consumption (Bevelhimer et al. 1985). The basic equation is

$$(1) \quad G = C - (R + F + U)$$

where $G$ = specific growth rate (grams per gram per day), C = specific rate of food consumption, $R$ = specific rate of respiration, which includes routine and active metabolism and heat increment components, $F$ = specific rate of egestion, and $U$ = specific rate of excretion. Most other bioenergetics models operate on daily or longer time steps, but a unique feature of this model is that it operates on 30-min time steps to more accurately reflect the time scale on which changes in thermal exposure and feeding occur as a result of vertical migration.

Figure 1 illustrates the general structure and operation of the model, the basic relationships among various model components, and the primary habitat variables (e.g., depth, temperature, and prey density) that influence foraging strategy. For each 24-h simulation, the initial input includes a prey/temperature array (i.e., a vertical profile of prey densities and temperature), the weight of the fish, the depths between which vertical migration occurs, the time spent at each depth, and the time of active feeding. The feeding status (feeding or not feeding) during the time step was also determined from the initial input. When feeding, the amount of food consumed was determined as a function of the food density at the fish's depth during that time step. Food intake was at times limited to less than the maximum rate when the stomach fullness was near capacity due to prior

TABLE 1. Equations used in the bioenergetics model for kokanee salmon. The sources of the original data and/or equations upon which these derivations were based include (1) Hyatt (1980), (2) Brett (1971b), (3) Dumont et al. (1975), (4) Richman (1958), (5) Hewett and Johnson (1987), (6) Elliott and Persson (1978), (7) Brett and Higgs (1970), (8) Brett and Glass (1973), (9) Brett and Groves (1979), and (10) Elliott and Davison (1975).

| Parameter | Source |
|---|---|
| **Consumption ($C$)** | |
| $C$ (prey/h) = $(Z / (0.0138 \cdot Z + 11.5)) \cdot 60$ | 1 |
| Stomach capacity of 100-g kokanee = $0.04 \cdot W$ | 2 |
| Stomach capacity of 500- and 1000-g kokanee = $0.022 \cdot W$ | 2 |
| *Daphnia* wet weight (mg) = $0.052 \cdot L_d^{3.012}$ | 3 |
| *Daphnia* dry weight (mg) = $1.23 \times 10^{-10} \cdot (L_d \cdot 1000)^{2.61}$ | 3 |
| *Daphnia* caloric content: 1 g wet weight = 586 cal | 4 |
| Kokanee caloric content: 1 g wet weight = $1362 + 0.736 \cdot W$ cal | 5 |
| **Digestion ($D$)** | |
| $D = W_{(0)} + C \cdot t - (W_{(0)} \cdot \exp(-rt)) + C/r \cdot (1 - \exp(-rt)))$ | 6 |
| Digestion coefficient: $r = 0.0154 - 0.0140 \cdot T$ | 7 |
| **Egestion ($F$)** | |
| $F = 0.455 \cdot T^{-0.222} \cdot D$ | 5 |
| **Excretion ($U$)** | |
| $U = 0.0233 \cdot T^{0.580} \cdot A$ | 5 |
| **Respiration ($R$)** | |
| Total respiration: | 8 |
| $R = \exp[\ln(R_{(std)}) + ((\ln(R_{(act)}) - \ln(R_{(std)})) \cdot S/\mathrm{MSS})] + \mathrm{SDA}$ | |
| Maximum sustainable swimming speed (cm/s): | 8 |
| $\mathrm{MSS} = \exp(0.909 + 0.632 \cdot \ln(L) - 0.00901 \cdot T + 0.00349 \cdot T^2 - 0.000128 \cdot T^3)$ | |
| Standard respiration (mg $O_2 \cdot g^{-1} \cdot h^{-1}$): | 8 |
| $R_{(std)} = \exp(-2.47 + 0.882 \cdot \ln(W) + 0.00126 \cdot T + 0.00286 \cdot T^2)$ | |
| Active respiration (mg $O_2 \cdot g^{-1} \cdot h^{-1}$): | 8 |
| $R_{(act)} = \exp(-1.19 + 0.986 \cdot \ln(W) + 0.111 \cdot T - 0.00294 \cdot T^2)$ | |
| Heat increment: $\mathrm{SDA} = 0.14 \cdot C$ | 9 |
| Oxycalorific conversion factor: 3.24 mg $O_2$ = 1 cal | 10 |
| where | Range |
| $Z$ = zooplankton density (no. /$m^3$) | 0–7358 |
| $W$ = kokanee wet weight (g) | 100–1000 |
| $L_d$ = *Daphnia* length (mm) | 2.25 |
| $W_{(0)}$ = weight (g) of food in the stomach at beginning of each time step | — |
| $t$ = length of model time step (h) | 0.5 |
| $T$ = temperature (°C) | 5–25 |
| S = swimming speed (cm/s) | 0–20 |

feeding, but stomach fullness was not used as a trigger to terminate or initiate feeding. The amount of food consumed was added to the amount of undigested food remaining in the stomach (if any) from the previous time step. The amount of food digested during a time step was calculated as a function of the amount of food in the stomach at the beginning of the time step plus the food intake during the time step. Since all the food that was consumed during a time step was not necessarily digested during that time step, we chose to use $D$, the amount of food digested (or processed), instead of C, the amount of food consumed, to represent the energy intake during a time step. Equation (1) thus becomes

$$(2) \quad G = D - (R + F + U).$$

A portion of the processed food is not useful to the fish and is egested as feces ($F$). Energy not lost in the feces is the amount of useful energy ($A$) available for assimilation, that is

$$(3) \quad A = D - F.$$

Of this assimilated energy, a portion is lost through excretion ($U$) as nitrogenous wastes. The last item to be subtracted as a cost is the respiration component which includes the metabolic costs associated with standard and active metabolism and heat increment (also known as specific dynamic action, SDA). Heat increment is the metabolic cost associated with the biochemical transformation of consumed food to usable energy forms. Subtracting the costs of egestion ($F$), excretion ($U$), and respiration ($R$) from the energy processed ($D$) resulted in the growth ($G$) or

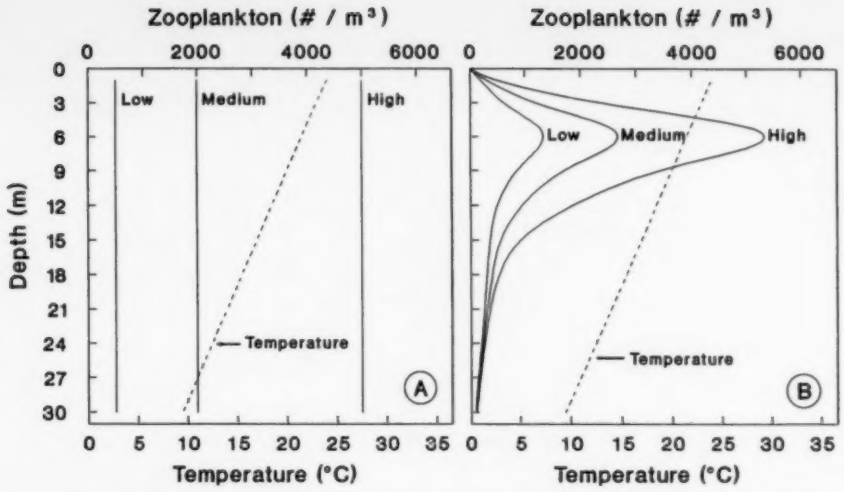



FIG. 2. (A) Uniform and (B) unimodal prey density distributions used in the bioenergetics model. Three levels of zooplankton densities (high, medium, and low) are represented by solid lines with temperature indicated by a broken line.

change in weight (either positive or negative) for the time step. The food remaining in the stomach was carried over to the next time step, and calculations were repeated beginning with the input of the depth occupied by the fish during the next time step. A summary of the equations and values used in the model is found in Table 1. For a more detailed description of the components and format of the model, see Bevelhimer (1990).

The model continued in 30-min time steps for 24 h. Change in weight for the entire 24 h was summed to provide an estimate of daily growth (grams per day). The simulation was repeated using the final stomach content value of the first 24-h run for the initial stomach content of the second run. This iterative process was repeated until a steady state was reached (i.e., the final stomach content was equal to the initial stomach content for that 24-h simulation). Normally, only two or three such iterations were necessary to achieve this steady state. A steady-state situation (equal initial and final stomach contents) would occur naturally if the 24-h feeding pattern of kokanee and the food density remained unchanged from day to day. In the long term, this is certainly not the case, but in the short term, this assumption is reasonable if in fact the feeding pattern is on a diel cycle. The results of the final steady-state simulation were those used in the analysis. The data derived from the simulations were daily estimates of consumption rate, energetic costs, growth, and gross conversion efficiency (kilocalories growth per kilocalorie consumed).

The entire model was constructed as a Lotus 1-2-3 spreadsheet which allowed for easy manipulation of parameters and provided immediate results from those manipulations. The depths between which vertical migration occurred could be entered manually (one at a time) or automatically with spreadsheet macros. Once the initial conditions (prey/temperature array, migration depths and times, fish weight, and feeding status) were entered, the calculations and iterations were performed automatically.

*Model simulations*

Simulations were designed to evaluate the behavior of the model or to test possible patterns of diel vertical migration. Various scenarios were established through the choice of the prey/temperature array and the migration pattern of the kokanee. The 13 prey/temperature arrays used in the model included six hypothetical arrays, six empirical arrays constructed from field data, and one projected array based on field data. The six hypothetical arrays were used to evaluate the basic behavior of the model and included high, medium, and low densities of prey distributed uniformly throughout the water column (Fig. 2A) and high, medium, and low densities of prey in an unimodal distribution with peak prey density at 6 m (Fig. 2B). For the six hypothetical arrays, the temperature stratification was represented as a linear decrease from 24.5°C at the surface to 9.5°C at 30 m; these arrays will be identified by their prey distribution (e.g., uniform-medium or unimodal-high). The arrays with the unimodal prey distribution are similar to natural situations in which the heterogeneity in the prey and temperature distributions produces the foraging dilemma discussed in the introduction. The six empirical arrays consisted of sampling data from June 1985 to June 1988 and were used to evaluate migration strategies under natural prey densities and temperature stratification; these arrays will be identified by their date of collection. The projected array included an estimated nighttime distribution of zooplankton based on the measured nighttime distribution on a different date.

Initial simulations were run without any vertical migration of the kokanee to demonstrate the relative values of the various depths in terms of food availability and energy conservation. These simulations were performed with the six hypothetical prey/temperature arrays.

A typical simulation was run with a combination of two depths, one when feeding and one when not feeding. These depth combinations were chosen to represent all reasonable patterns of diel vertical movements. That is, a fish fed at a particular depth and then remained at that depth or moved to a lower depth when not feeding. Only depths that were multiples of 3 m were used in most simulations. For example, a fish modeled at 3 m when feeding would be at either 3, 6, 9, . . ., or 30 m when not feeding, and so on for all possible feeding depths. Simulations were

TABLE 2. Variables used in simulations of the vertical migration of kokanee salmon. The conditions of the standard simulation are underlined.

| Variable | Range of values |
|---|---|
| Fish size | 100, 500, or 1000 g |
| Feeding duration | 8, 10, 12, or 16 h/d |
| Feeding swim speed | 20 or 30 cm/s |
| Feeding pattern | Diurnal or nocturnal |

normally limited to a maximum depth of 30 m, which was near the maximum depth of the reservoir in the upper half of the reservoir where kokanee were normally captured and water quality measurements were made. For each prey/temperature array, a series of up to 55 simulations was performed to determine the optimal migration strategy for a fish of a given size with a particular temporal feeding pattern.

The standard simulation consisted of a 500-g kokanee, with a diurnal feeding pattern of 12 h of feeding per day, and the unimodal–high array. Kokanee size, feeding duration, swimming speed, and feeding pattern were varied one at a time in other simulations to evaluate their respective effects on the predictions of the optimal migration strategy. The feeding pattern was usually diurnal, but a nocturnal pattern was also simulated. Table 2 summarizes those variables that were manipulated in the simulations. Simulations with the six empirical arrays were the same as the standard simulation except for the substitution of one of the empirical arrays for the hypothetical array.

The results of the modeling exercise were compared with the migration patterns observed during the hydroacoustic surveys. To facilitate this comparison, simulations using the prey/temperature array collected during the 1988 hydroacoustic survey were modified to reflect the observed kokanee behavior during the survey. These modifications included a smaller kokanee size (100 g) and a different feeding pattern (nocturnal instead of diurnal) with the projected nighttime zooplankton distribution. The projected nighttime zooplankton distribution was estimated using the same distribution shape as that observed during night sampling in June 1985 and then scaling it to the peak daytime density. Feeding at night by kokanee has been documented (Northcote and Lorz 1966), and feeding by sockeye at light levels less than bright moonlight (0.032 lx) has also been observed (Brett and Groot 1963).

## Results

Temperature data from six dates were used in the model (Fig. 3). The temperature profile of Nantahala Lake can generally be characterized as gradually decreasing from the surface downward, usually with no distinct thermocline. Because of the altitude and depth of Nantahala Lake, surface and bottom temperatures during the summer are cooler than most reservoirs in the southeastern United States. DO concentrations exceeded 7.0 ppm throughout the vertical profiles on four of the six dates. On the other two dates (July 1987 and October 1985), DO was <7.0 ppm below 9 and 24 m deep, respectively. The majority of the kokanee captured on these two dates were at depths where DO < 7.0, suggesting that even though there is evidence that Pacific salmon avoid DO < 7.0 ppm (Birtwell and Kruzynski 1989), these levels do not appear to be absolutely limiting. Secchi depths were normally in the 3- to 5-m range. Light profiles of the water column indicated that at midday the intensity at 30 m is approximately 1.1 lx. Brett and Groot (1963) found that juvenile salmon could feed at maximum rates at this intensity or higher, suggesting that light should not be a limiting factor on feeding to a depth of at least 30 m during midday. They also reported that at total darkness ($<1.1 \times 10^{-4}$ lx) the salmon did not feed even at high prey densities.

For vertical profiles of zooplankton densities (Fig. 3), linear interpolation provided values at depths that were not sampled on the six dates. Values at the surface and 30 m were projected to be zero to provide a complete profile of the prey density for the model simulations. *Daphnia* sp. accounted for nearly 95% of the larger zooplankton (>1 mm) in the lake with *Leptodora*, chironomid larvae and pupae, *Chaoborus*, *Holopedium* sp., and copepods making up smaller proportions of this size class of zooplankton. Large zooplankton were generally more abundant in late spring and early summer than in late summer and fall. Smaller zooplankton (<1 mm) consisted mainly of copepods, *Daphnia* sp., and *Bosmina* sp., but were not quantified.

Kokanee (180–550 mm total length, 51–1459 g wet weight) were captured over a wide range of depths (5–29 m) and temperatures (7–22°C) during the study. However, most (87%) were captured in an 8°C range (7.5–15.5°C) with a mode at 12°C (Fig. 4). The precise time of entanglement (i.e., late evening, during the night, or early morning) is unknown, although entanglement during the night was observed on one occasion when nets were checked throughout the night. Seasonal changes in kokanee distributions reflected the seasonal changes in the thermal profile of the lake. As the summer progressed and water temperatures warmed, they were captured at increasingly greater depths. Kokanee were captured at temperatures up to 22°C which indicates that some occasionally move into water that is much warmer than their preferred temperature range. No obvious size- or sex-related differences in depth or temperature of capture were noticed.

Species other than kokanee (rainbow trout, *Oncorhynchus mykiss*, channel catfish, walleye, black crappie, *Pomoxis nigromaculatus*, white crappie, *Pomoxis annularis*, rock bass, *Ambloplites rupestris*, redbreast sunfish, *Lepomis auritus*, and white sucker, *Catostomus commersoni*) composed approximately 50–65% of the total catch. Except for rainbow trout, which were captured at 16–20°C (Fig. 4), little overlap in depth/temperature distribution occurred between kokanee and the other species.

As reported by others, the diets of the kokanee consisted exclusively of zooplankton and included *Daphnia* sp., chironomid pupae and larvae, *Chaoborus* sp. larvae, and *Leptodora* sp. Large *Daphnia* (mean size approximately 2.25 mm) accounted for at least 95% of items in 55 of the 58 stomachs with identifiable prey.

Results of an intensive 24-h hydroacoustic survey indicated that adult kokanee were possibly undergoing limited vertical migrations in late July 1987 (Fig. 5A). Rainbow trout and kokanee were captured in gill nets at depths of 0–21 and 16–26 m, respectively, during this sampling trip. Given the depth and temperature range differences between these two species, the high temperatures (>25°C) in the upper 5 m, and general knowledge of the habitat preferences (particularly thermal) of the other species in the system, the following assumptions were made during the interpretation of the hydroacoustics output. Fish in the upper 8 m of the water column were assumed to be primarily channel catfish, rainbow trout, or other species, but not kokanee; fish from 8–20 m could conceivably have been rainbow trout or

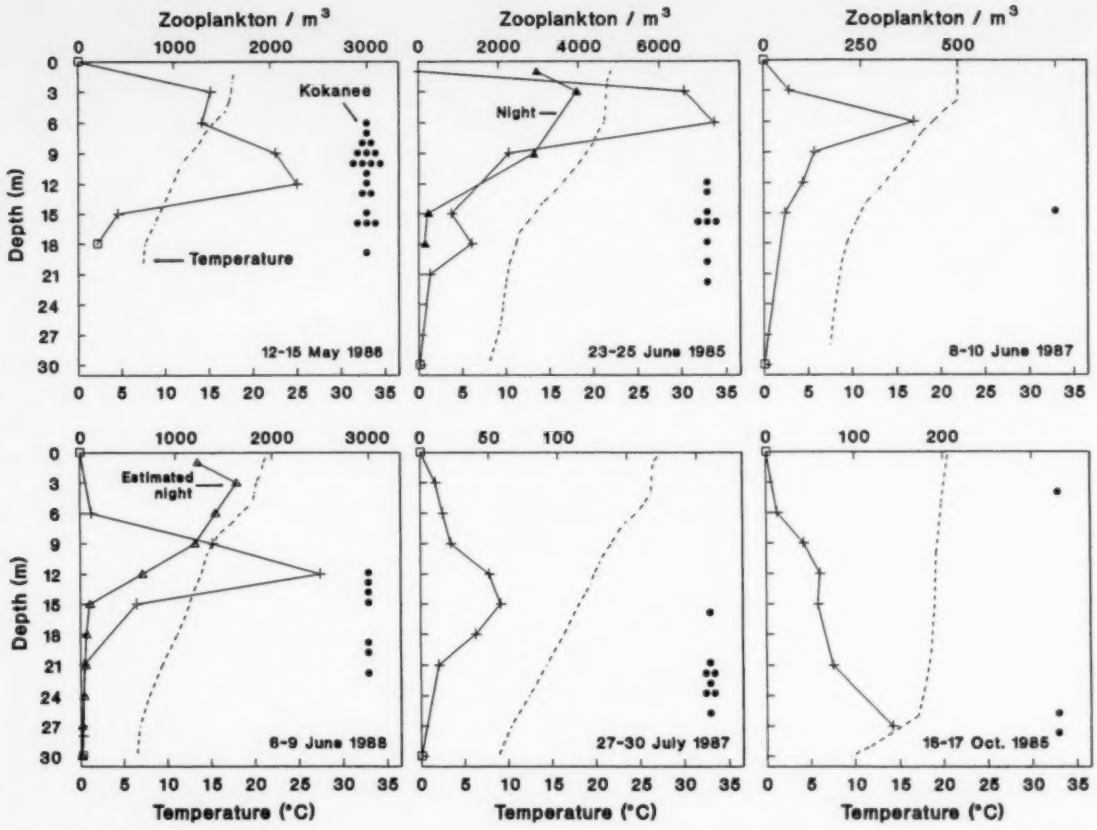



FIG. 3. Vertical profiles of zooplankton >1 mm (solid lines) and temperature (broken lines) measured in Nantahala Lake on six occasions. Plus signs represent measured densities from horizontal zooplankton tows, and open squares represent projected densities for use in the bioenergetics model when actual values were not available. All zooplankton profiles represent daytime distributions except for two nighttime distributions, one observed (solid triangles) and one projected (open triangles). Solid circles indicate the depths of individual kokanee salmon captured in gill nets on the same sampling trip.

kokanee; and fish from 20–36 m were most likely kokanee. Given these species-specific differences in depth distribution, the hydroacoustic survey results were interpreted as follows. At dawn (sample start time 05:58), kokanee were most likely between 20 and 32 m, but some may also be at 8–16 m. By midday (13:10) through late afternoon (17:10), they might be anywhere from 8 to 24 m. At dusk (20:25), kokanee were most likely between 14 and 24 m. After dark, the kokanee appear to move deeper to 20–32 m by 22:00 and 22–32 m by 23:55. Although it is impossible to tell what individuals are doing, the population as a whole appears to be between 8 and 24 m during the day (zooplankton densities were highest at 11–18 m, see Fig. 3) and between 20 and 32 m during the night. The kokanee appear to be distributed over a greater depth range around sunrise and sunset than during the middle of the night or day. That the kokanee were usually spread over an 8-m or greater range in the water column may be an indication of the degree of precision (or lack thereof) with which vertical position is selected. This also suggests a fair amount of individual variation and/or asynchrony in the timing of movements.

In June 1988, reconnaissance surveys prior to the intensive 24-h survey were unable to locate any fish resembling adult kokanee, and no adult kokanee were captured in the nets at this time. A concentration of small fish (presumably juvenile kokanee) were located at depths >40 m during afternoon reconnaissance surveys. In addition, juvenile kokanee (179–197 mm) were captured in the nets. Unlike the previous year when surface temperatures during the hydroacoustic survey exceeded 25°C, the temperature at 1 m was only 21°C; therefore, fish marked in the upper few metres of the water column were not ruled out as kokanee. The results of this survey (Fig. 5B) were interpreted as follows. Although the targets from 2–10 m could be several species, the fish at 42–50 m in the afternoon (14:35) are almost certainly kokanee. Only three fish were marked from 10–42 m at this time. At dusk (20:50), these kokanee had left the deepest water and moved to shallower than 30 m. Throughout the night (21:25, 22:05, and 00:05), most targets were at 2–24 m with a few from 24–50 m. The overnight gillnet sets during this same sampling trip support this observation — juvenile kokanee were captured at 13–22 m. At dawn (05:40), most of the fish were observed at 24–42 m. After sunrise (06:30), most were at 30–42 m, and by late morning (10:55) the concentration of fish at 42–50 m was reestablished. Given this interpretation, the kokanee are migrating from 42–50 m to at least 24 m (an average

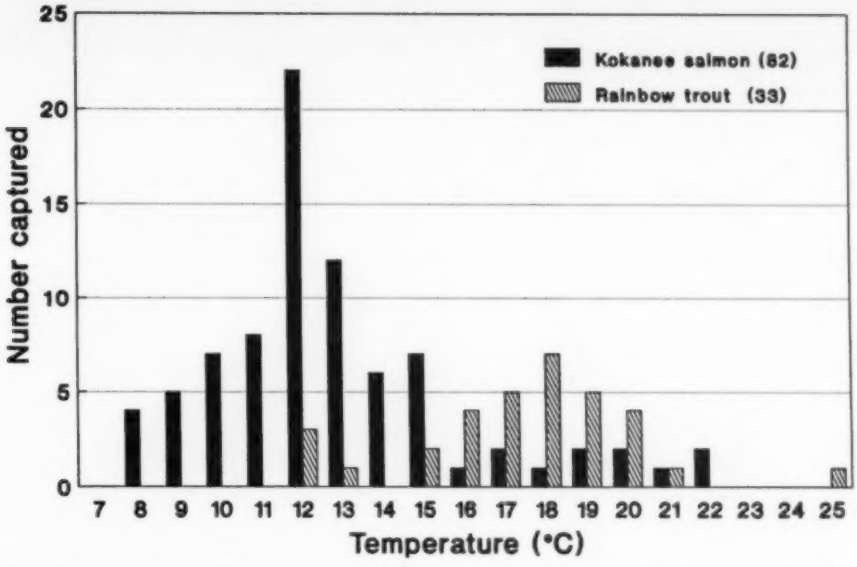



FIG. 4. Temperature distributions of 82 kokanee salmon (180–550 mm) and 33 rainbow trout (277–518 mm) captured in gill nets during 10 sampling trips from 1985 to 1988 in Nantahala Lake.

migration of 22 m) and possibly as far up as the surface (>40-m migration). The conspicuous absence of large numbers of fish at the 2- to 6-m range during the dawn (05:40 and 06:29) and dusk (20:48) surveys is difficult to explain. This could possibly be a result of offshore-inshore migrations by species other than kokanee or movements to the top 2 m where targets are not detected by this hydroacoustic technique.

## Model Simulations

In simulations with the uniform–high array (Fig. 2A) and no vertical migration, growth for a 500-g kokanee was optimal at 18 m (15.5°C) (Fig. 6A). Growth was lower at depths <18 m due to increased metabolic rates associated with higher temperatures. At depths >18 m, growth was inhibited because food consumption was limited by slower digestion rates associated with colder temperatures. That is, because prey were abundant and feeding rate was high, the stomach was filled in a few hours, after which time the feeding rate was limited by the rate at which the stomach was evacuated. The depth of optimal growth, however, was not the depth of greatest gross conversion efficiency which occurred at 27 m (11°C). With the uniform–medium array, the effect of the digestion rate/stomach capacity interaction was only important at 27 and 30 m because of a decrease in the feeding rate, and the depth of optimal growth was therefore deeper (24 m, 12.5°C) than when food was more abundant. With the uniform–low array, the limitations on food consumption by digestion rate and stomach capacity were not important, and optimal growth was achieved at 30 m (9.5°C) where metabolic costs were minimal.

In simulations with the unimodal prey distributions (see Fig. 2B) and no vertical migration, the resulting growth-at-depth (Fig. 6B) was more reflective of the prey distribution than simulations with the uniform prey distributions. With the unimodal–high array, maximum growth occurred at 9 m (20°C) even though the greatest prey density (and the highest feeding rate) occurred at 6 m (21.5°C). The increased food consumption at 6 m as compared with 9 m was offset by an even greater total energetic cost at 6 m due to warmer temperatures. In addition, the difference in daily consumption of fish between 6 and 9 m was not of the same magnitude as the difference in prey densities due to the effects of the physical limitations of feeding rate and stomach capacity. The most efficient depth for growth with the high prey densities was 12 m (18.5°C) where gross conversion efficiency was 28%. With the unimodal–medium and unimodal–low arrays the depth of maximum growth was 6 m. At lower levels of food availability and consumption, stomach capacity does not have the same limiting effects as at higher prey densities; therefore, the growth-at-depth relationship more closely reflects the prey distribution.

When diel vertical migration was included in the model, standard simulations with the unimodal–high array generated growth rates ranging from –0.51 to 3.28 g/d (Fig. 7). The most obvious result of these simulations is that there is a definite advantage to some type of vertical migration given this prey/temperature array. Although the difference in growth among the 10 most productive migration patterns is slight, these patterns are similar and can be collectively summarized as feeding from 6 to 9 m and then moving to 15–30 m when not feeding. Since the greatest growth occurred with a nonfeeding depth of 30 m, migration to a greater depth with cooler temperature would probably produce greater growth until digestion rates became slow enough to inhibit the quantity of food that could be consumed in future feedings.

Because differences among the top five or 10 most productive migration strategies were slight, in the analyses that follow the five migration patterns with the highest growth will be presented for evaluation instead of just the single migration pattern that produced the highest growth.

The effect of prey density was evaluated with the three hypothetical levels of unimodal prey distributions (Fig. 2B). A fourth level was created by doubling the consumption rate at the

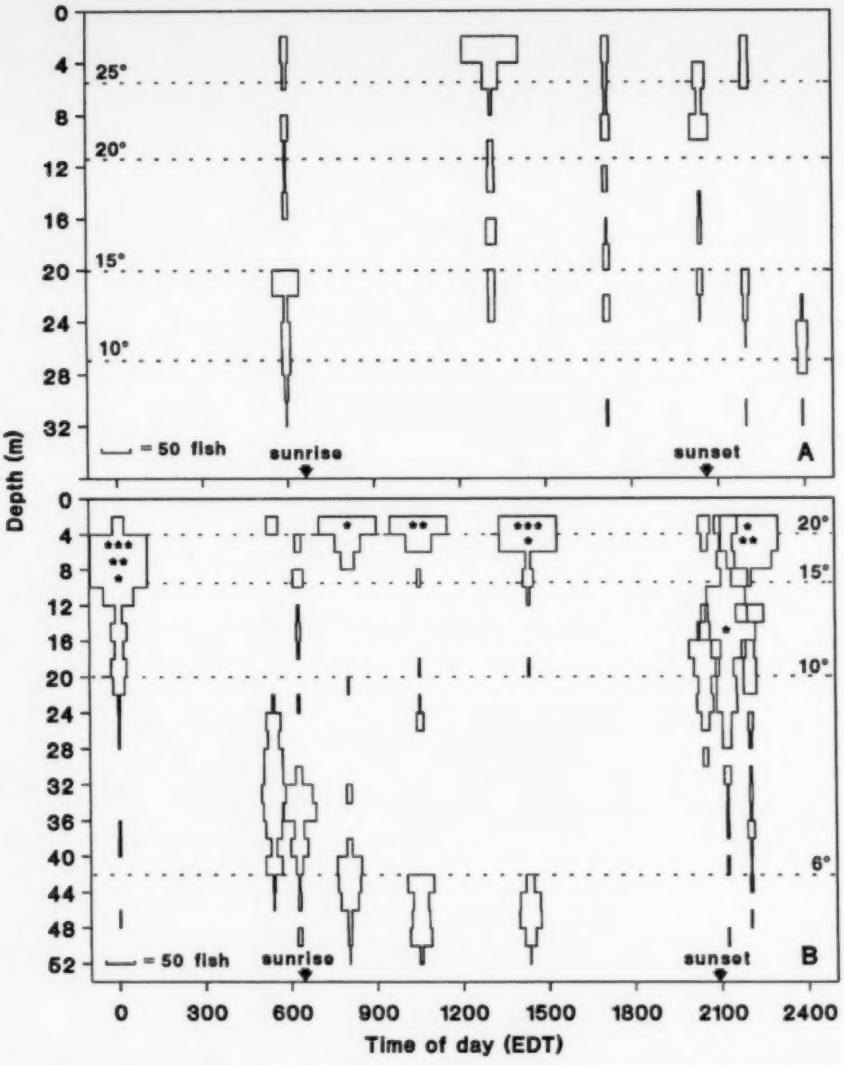



FIG. 5. Number of fish located (represented by the width of each block) at each 2-m depth interval during hydroacoustic surveys on (A) 28–29 July 1987 and (B) 7–8 June 1988. Intervals that exceeded 100 fish are marked with asterisks: *101–150, **151–200, and ***201–250 fish, respectively. The fish counted were those registering a target strength of −24 to −38 dB (comparable to adult-sized kokanee) and −39 to −59 dB (comparable to juvenile-sized kokanee) in 1987 and 1988, respectively. Horizontal dotted lines indicate temperatures.

high level. The results indicate that, generally, the best strategy is to move to where the food is most abundant to feed and then to the coolest water when not feeding (Fig. 8A) as long as the water is not so cold as to slow digestion to the point that subsequent feeding bouts are limited by leftover food in the stomach. The differences in growth were as expected — higher levels of prey resulted in greater growth. The advantages of migration as indicated by a comparison of the resulting growth versus the growth achieved when not migrating are most important in the simulation with low prey density and least important with the highest density (Fig. 8A).

The effect of the amount of time spent feeding each day was evaluated at foraging durations of 4, 8, 12, and 16 h/d with the standard setup. At 4 h/d, feeding at either 6 or 9 m was most productive (Fig. 8B). However, as feeding time increased, feeding at 6 m alone produced the highest growth rates. The difference in growth is as expected — those that feed for a longer time grow more. The growth advantages of migration are most important at the shorter feeding durations (Fig. 8B).

The effect of fish size on optimal strategy was evaluated for fish of 100, 500, and 1000 g with other variables at the standard values. These results suggest that as size increases, the upper limits of migration should be slightly deeper (Fig. 8C). This pattern was largely influenced by weight-specific differences in

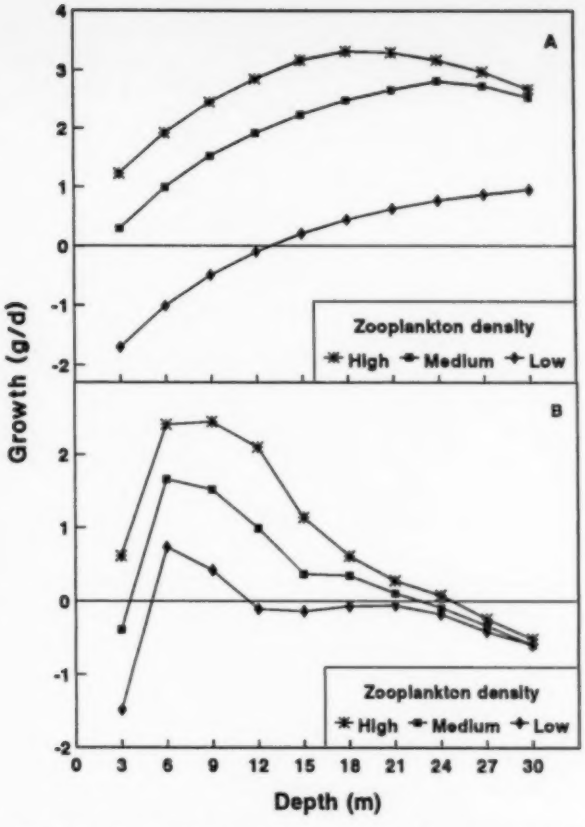



FIG. 6. Results of model simulations of growth at three prey densities with (A) uniform and (B) unimodal vertical distributions when no vertical migration of kokanee was simulated.

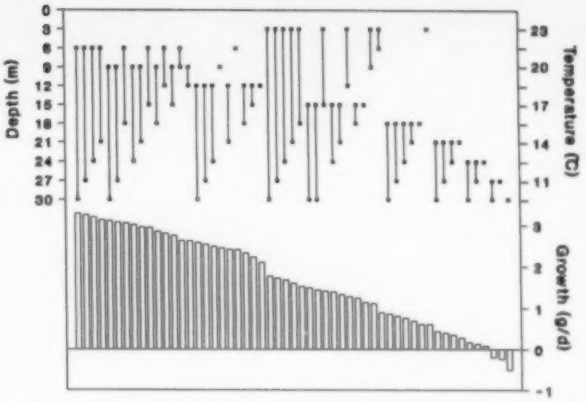



FIG. 7. Results of standard model simulation. Top half of graph indicates the combination of depths between which vertical migration occurred (no movement is indicated by a single point). Each histogram at the bottom indicates the growth resulting from the migration pattern directly above.

metabolic rate and stomach capacity. The advantages of migration are small for the 100-g fish, but become markedly more important as size increases (Fig. 8C).

The effect of swimming speed (i.e., increase in active metabolism) on the migration strategy was evaluated by increasing the swimming speed during feeding from 20 to 30 cm/s. An additional simulation was performed to allow for an increase in consumption of the same magnitude (50%) as the increase in swimming speed, assuming that search volume and thus consumption increases proportionally to swimming speed. With changes in swimming speed alone, the basic migration strategy remained the same, but growth was lower at the higher swimming speed because of increased metabolic costs. Increasing consumption along with swimming speed resulted in the same general migration patterns being most productive. Growth in this instance (with increased speed and consumption) was greater than when the fish was at the "normal" swimming speed and rate of consumption.

The standard simulation was also performed with the six empirical prey/temperature arrays to evaluate seasonal variation in optimal migration strategy (Fig. 8D). The model predicted that under spring (May 1986) conditions, vertical migration would be limited or nonexistent and confined to the middle of the water column. With late spring and early summer (June 1985, 1987, 1988) conditions, the importance of vertical migration (as indicated by the resulting growth) increased, and the predicted range of migrations might be as great as 24 m. For midsummer (July 1987) and fall (October 1985) conditions, the model predicted that vertical migrations were of little value and that kokanee would select fairly deep locations.

The modified June 1988 simulations (100-g kokanee feeding at night) predicted that ascending to 3 m to feed and descending to 9–21 m when not feeding was the most energetically efficient. The model predicts higher growth and different optimal migration patterns when feeding at night than during the day (Fig. 9).

## Discussion

In general, predictions of the bioenergetics model are consistent with the expected growth dynamics of salmonids as evidenced by consumption rates that were about 4.5% body weight per day, growth rates about 1% body weight per day, and gross conversion efficiency of about 35%. All of these are within the ranges expected for this size of fish when feeding at a high ration (Brett 1979).

The modeling results demonstrate that when the preferred temperature and optimum prey density are spatially separated, vertical migration can result in greater growth compared with no migration. Because of the interaction of factors such as feeding rate, stomach evacuation rate, stomach capacity, and respiration rate, the most productive location for feeding is not always the one with the greatest food density. Likewise, the most productive location for resting is not always that where metabolic costs are minimized. In addition the combination of depths that produces the highest growth rate is usually not the same as the combination that produces the highest conversion efficiency. Greater growth can sometimes be achieved by processing more food at a lower efficiency than by processing less food at maximum efficiency.

Levy (1990a) minimized the importance of the energetic benefits of vertical migration based on the assumption that such benefits would only be realized when a substantial temperature difference existed between selected vertical positions. He concluded that the mean difference of 4°C that he observed would result in only minor growth differences. In fact, based on the

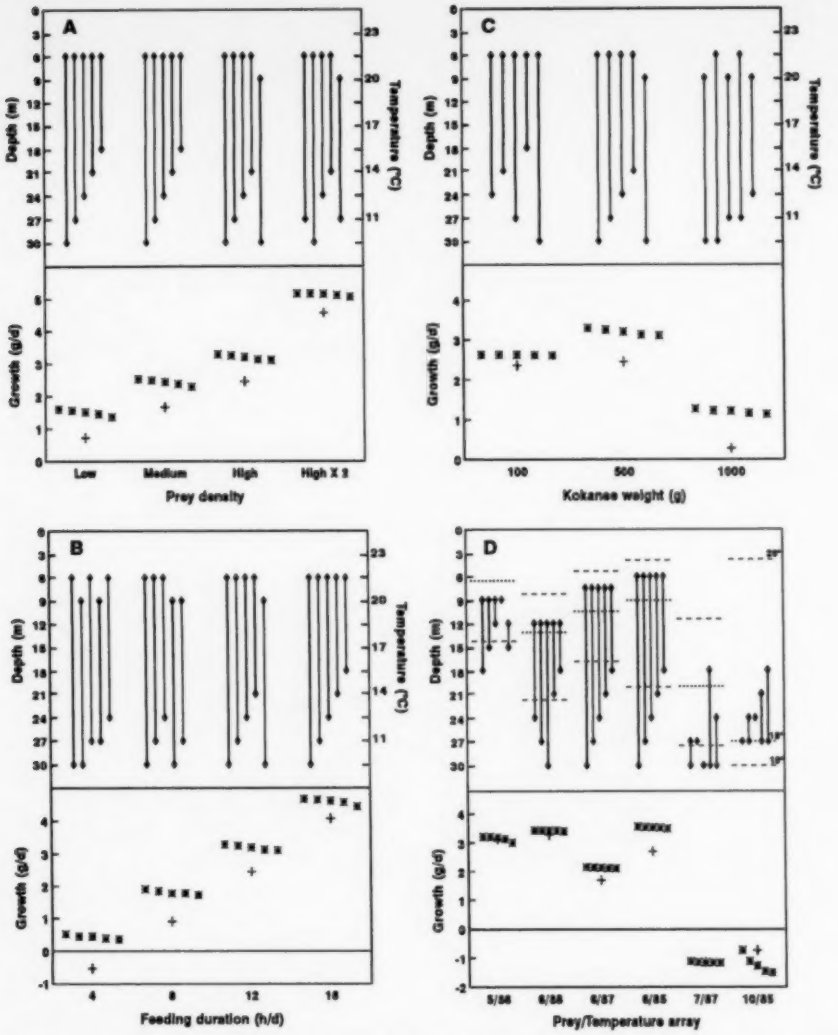



FIG. 8. Five migration patterns (upper panels) which resulted in the highest simulated growth (lower panels, in descending order left to right) for (A) each of four treatments of prey density, (B) each of four treatments of feeding duration, (C) each of three treatments of fish weight, and (D) each of six actual prey/temperature arrays from May 1986, June 1985, June 1987, June 1988, July 1987, and October 1985. In Fig. 8D, the 20°C depth is indicated by the top broken line, the 15°C depth by the dotted line, and the 10°C depth by the bottom broken line. Growth from each migration pattern indicated in the upper panels is indicated with an asterisk. Growth of the highest ranking nonmigration pattern (plus sign) is also included for comparison regardless of its overall rank.

results of the standard simulation (see Fig. 7), a difference of 4.5°C (21.5–17.0°C) from the feeding depth (6 m) to the nonfeeding depth (15 m) resulted in a 21% increase in growth rate over the nonmigration alternative of staying at 6 m. A more substantial temperature difference (9°C from 6 to 24 m) during vertical migration resulted in a 33% increase in growth rate over not migrating. Even a 21% difference in growth could have a substantial impact on size-dependent processes such as predation mortality, egg production, and mate selection.

Several assumptions within the model should not be overlooked in its evaluation. The effect of the time lag between a change in ambient water temperature and the body core temperature on respiration and other physiological functions was assumed to be negligible with regards to the predictions of the model because over the course of a day, both heating and cooling of the same magnitude are experienced. Although Weller et al. (1984) found that heat exchange occurred at a faster rate when heating than when cooling, these differences are not expected to have a noticeable effect on the predictions of vertical migration strategy.

Another important assumption in the model was that there was no additional metabolic cost incurred due to vertical migration other than a change in respiration rate related to the change in temperature and swimming speed that accompanied each vertical

migration. As a fish moves up or down in the water column, its buoyancy changes due to changes in hydrodynamic pressure. A fish that is not at neutral buoyancy must compensate by active movement or by deflation or inflation of the swim bladder to remain at the same depth. Alexander (1972) estimated the additional cost of swimming activity needed to compensate for negative buoyancy by a shallow-acclimated fish that migrates to deeper water as approximately 25 $cm^3$ $O_2 \cdot kg^{-1} \cdot h^{-1}$ (36 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) while at the lower depth. Clark and Levy (1988) used this value to refute the energetic advantages of vertical migration. For the standard simulation in this study, this additional cost becomes about 0.7 kcal/d which would reduce the daily growth by about 20%. This is possibly an overestimate for kokanee because the swimming activity associated with feeding should account for much of the activity required to maintain position. Even with this reduction, vertical migration is still a more productive strategy than not migrating at all. Alexander (1972) also estimated the cost of swim bladder inflation for buoyancy compensation as approximately 10 times less costly than active compensation. Although compensation via swimbladder volume control is the more economical, this method may not occur fast enough in salmonids to maintain a steady volume at the rate of depth change that has been observed. Unfortunately, the type of data required to make accurate calculations of the energetic costs of vertical migration of kokanee (or any other species) are not available. Even Alexander (1972) stated that there is so much uncertainty in the data that it is difficult to make firm conclusions about the energetic advantages of vertical migration based only on the physical costs of vertical migration.

Due to a lack of information, it was also assumed that all sizes of kokanee had the same feeding rate relative to prey density. This assumption had some effect on the predictions of vertical migration strategy, but is more critical to accurate predictions of growth. Information regarding differences in maximum feeding rate due to size for particulate-feeding fish is scarce. Certainly, fish can increase their effective search volume by increasing their swimming speed, thus increasing their encounter rates, and large fish should be able to sustain increased swimming speeds for longer periods than small fish. Therefore, we might expect larger fish to have higher feeding rates especially at lower prey densities. At high prey densities, increasing the encounter rate may not make much difference if the limiting factor is handling time. In this model the swimming speed while foraging was also the same for all sizes. This assumption is reasonable, considering that feeding rates were the same for all sizes for any given prey density, but it would be more realistic to use size-dependent and prey-density-dependent relationships to determine feeding rate and swimming speed if this information were available.

Sensitivity analyses were performed on several variables in the model to determine their relative importance to model predictions. Changes in the swimming speed within the ranges tested did not noticeably affect the migration predictions. Varying the prey density from low to high also did not result in different predictions of optimal migration strategy. However, had the shape of the prey distribution been changed, optimal migration patterns probably would have differed.

Small changes in the optimal migration patterns were predicted when the feeding time was varied from 4 to 16 h. As feeding time increased, consumption increased and migration priority seemed to shift from one of metabolic cost minimization (i.e., residing at the deepest depths, 24–30 m) to one of maximizing food intake (i.e., feeding where a combination of high

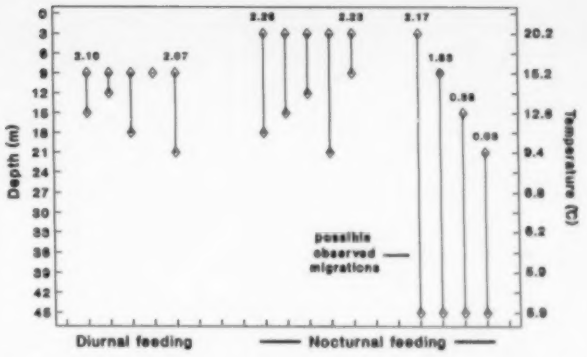



FIG. 9. Five migration patterns which resulted in the highest simulated growth (in descending order left to right) for 100-g kokanee with observed daytime (left side) and hypothetical nighttime (middle) prey distributions for June 1988. On the right are four possible migration patterns resulting from simulations (using the hypothetical nighttime prey distribution) as suggested by the hydroacoustics data. Corresponding growth (g/d) is included above some migration patterns.

food concentration and high digestion rate results in the maximum food consumption rate, 6 m).

Similar changes were predicted when fish size was varied. Because the prey consumption/prey density relationship was the same for all sizes of fish, the 1000-g fish consumed less relative to its size than did the 100-g fish. This resulted in different migration priorities as above. The priority for the 100-g fish was to maximize food intake, since it was able to consume at a high relative rate, while the priority for the 1000-g fish was to minimize metabolic costs when not feeding.

When simulations were performed with actual prey/temperature distributions, a seasonal variation in migration strategy was predicted. In spring when water temperatures in the upper half of the water column were still relatively cool (<20°C) and a significant thermal stratification had not yet developed, migrations of 0–9 m were predicted. As temperatures warmed and thermal stratification progressed in late spring and early summer, the benefits of vertical migration were greater and the range of migrations encompassed most of the water column. And by late summer and early fall when prey densities were greatly reduced, the benefits of vertical migration disappeared. Narver (1970) observed similar seasonal shifts in the magnitude of vertical migrations. Unfortunately, no information regarding migration patterns could be obtained from our net data, since the precise time of capture of individual fish could not be determined. However, the locations of kokanee captured in the nets (see Fig. 3) rarely exceeded the range of occurrence predicted by the model (Fig. 8D) for each of the six dates.

Comparisons of the simulations for July 1987 and June 1988 with the results of the hydroacoustic surveys performed on those dates are difficult because of the uncertainty in the interpretation of the hydroacoustics results. With the July 1987 data, the model predicted that kokanee should undergo only limited migrations or none at all due to low prey densities and warm temperatures in the upper half of the water column (see Fig. 8D). Such patterns are not ruled out by the hydroacoustics results, but are not supported either.

An evaluation of the 1988 hydroacoustic survey with the simulation results raises two questions. First, what are the energetic differences between diurnal and nocturnal feeding? The

resulting growth of the nocturnal simulations was slightly greater than that of the diurnal simulations (Fig. 9) even though the peak prey density was less during the night than during the day. Of course, this assumes that they can feed at the same rate during the night as during the day. Lower growth occurred during the day because the amount of food that could be processed at the depth of highest prey concentration (12 versus 3 m at night) was limited by the cooler temperatures (6°C less than at 3 m). Second, how does the observed migration pattern compare with the predictions of the model? The top five migration patterns predicted by the modified June 1988 simulations (Fig. 9) do not match the pattern observed during the hydroacoustic survey. The model predicted feeding at 3 m, which is not necessarily ruled out by the survey results, but also cannot be confirmed. The lower limits of migration predicted by the top five simulations were 9–21 m, which is much shallower than the observed lower limits of 42–50 m. However, the decrease in simulated growth associated with descending to the observed depths (i.e., migrating from 3 to 42–50 m) compared with the top ranked migration pattern (i.e., 3–18 m) is minimal — only a 4% decrease in growth. If the extent of the kokanee's upward migration is only 21 m, the predicted growth is 0.03 g/d — a 99% decrease from the optimal and not nearly enough to achieve normal growth. Although predator avoidance is a reasonable explanation for juvenile kokanee to feed at night and to descend to great depths during the day, these results suggest that there can be energetic advantages associated with nocturnal feeding as well.

We have demonstrated that bioenergetic differences among various vertical migration scenarios under a variety of conditions affect the growth advantage of a particular behavior. Whether vertical migration results in an energetic advantage depends on the size of forager, seasonal and daily variation in temperature profiles and prey distributions, consumption limitations, etc. Because of the variety of factors involved, vertical migration may vary widely among species and systems. In addition, vertical migration need not always be between areas of optimal thermal conditions and warmer than optimal conditions to be energetically advantageous. Energetic benefits may also be realized from migrating in and out of colder than optimal conditions (Wurtsbaugh and Neverman 1988). Lastly, growth maximization (or energetic efficiency) is not the only factor driving vertical migration. However, even when another factor (such as avoidance of predators) is of key importance, it seems reasonable to expect that energetic considerations still influence the vertical migration that is exhibited within the constraints of the primary factor. Ultimately, a complete understanding of vertical migration may necessitate a multifactor approach with individual factors receiving different value depending on seasonal and ontogenetic considerations.

## Acknowledgements

We thank the Environmental Sciences Division of Oak Ridge National Laboratory for providing necessary field equipment, the Tennessee Valley Authority (in particular R. Pasch, D. Lowery, and W. Wilson) for providing the hydroacoustic equipment and technical assistance, countless volunteers for assistance in the field, and L.J. Gross, D.L. DeAngelis, W. Wurtsbaugh, J. Janssen, and an anonymous reviewer for critically reviewing and improving the manuscript. This research was supported by the Graduate Program in Ecology at The University of Tennessee, Knoxville, and the laboratory graduate participation program of Oak Ridge Associated Universities under contract No. DE-AC05-76R00033 with the U.S. Department of Energy, and the Office of Health and Environmental Research, U.S. Department of Energy, under contract No. DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

## References

ALEXANDER, R.M. 1972. The energetics of vertical migration by fishes. Symp. Soc. Exp. Biol. 26: 273-294.

BEVELHIMER, M.S. 1990. Habitat selection by kokanee salmon and smallmouth bass in thermally heterogeneous environments: the importance of growth maximization to diel habitat shifts. Ph.D. thesis, University of Tennessee, Knoxville, Tenn.

BEVELHIMER, M.S., R.A. STEIN, AND R.F. CARLINE. 1985. Assessing significance of physiological differences among three esocids with a bioenergetics model. Can. J. Fish. Aquat. Sci. 42: 57–69.

BIRTWELL, I.K., AND G.M. KRUZYNSKI. 1989. *In situ* and laboratory studies on the behavior and survival of Pacific salmon (genus *Oncorhynchus*). Hydrobiologia 188/189: 543–560.

BRETT, J.R. 1971a. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11: 99–113.

BRETT, J.R. 1971b. Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 409–415.

BRETT, J.R. 1979. Environmental factors and growth, p. 599–675. *In* W.S. Hoar, D.J. Randall, and J.R. Brett [ed.] Fish physiology. Vol. 8. Academic Press, New York, N.Y.

BRETT, J.R., AND N.R. GLASS. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

BRETT, J.R., AND C. GROOT. 1963. Some aspects of olfactory and visual responses in Pacific salmon. J. Fish. Res. Board Can. 20: 287–303.

BRETT, J.R., AND T.D.D. GROVES. 1979. Physiological energetics, p. 279–352. *In* W.S. Hoar, D.J. Randall, and J.R. Brett [ed.] Fish physiology. Vol. 8. Academic Press, New York, N.Y.

BRETT, J.R., AND D.A. HIGGS. 1970. Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, *Oncorhynchus nerka*. J. Fish. Res. Board Can. 27: 1767–1779.

CLARK, C.W., AND D.A. LEVY. 1988. Diel vertical migrations by juvenile sockeye salmon and the antipredation window. Am. Nat. 131: 271–290.

DUMONT, H.J., I. VAN DE VELDE, AND S. DUMONT. 1975. The dry weight estimate of biomass in a selection of cladocera, copepoda and rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19: 75–97.

EGGERS, D.M. 1978. Limnetic feeding behavior of juvenile sockeye salmon in Lake Washington and predator avoidance. Limnol. Oceanogr. 23: 1114–1125.

ELLIOTT, J.M., AND W. DAVISON. 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19: 195–201.

ELLIOTT, J.M., AND L. PERSSON. 1978. The estimation of daily rates of food consumption for fish. J. Anim. Ecol. 47: 977–991.

HEWETT, S.W., AND B.L. JOHNSON. 1987. A generalized bioenergetics model for fish growth for microcomputers. University of Wisconsin Sea Grant Institute Publ. WIS-SG-87-245, Madison, Wis.

HORAK, D.L., AND H.A. TANNER. 1964. The use of vertical gill nets in studying fish depth distribution, Horsetooth Reservoir, Colorado. Trans. Am. Fish. Soc. 93: 137–145.

HYATT, K.D. 1980. Mechanisms of food resource partitioning and the foraging strategies of rainbow trout (*Salmo gairdneri*) and kokanee (*Oncorhynchus nerka*) in Marion Lake, British Columbia. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

JANSSEN, J., AND S.B. BRANDT. 1980. Feeding ecology and vertical migration of adult alewives (*Alosa pseudoharengus*) in Lake Michigan. Can. J. Fish. Aquat. Sci. 37: 177–184.

KITCHELL, J.F., D.J. STEWART, AND D. WEININGER. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 34: 1922–1935.

LEVY, D.A. 1987. Review of the ecological significance of diel vertical migrations by juvenile sockeye salmon (*Oncorhynchus nerka*), p. 44–52. *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

LEVY, D.A. 1990a. Reciprocal diel vertical migration behavior in planktivores and zooplankton in British Columbia lakes. Can. J. Fish. Aquat. Sci. 47: 1755–1764.

LEVY, D.A. 1990b. Sensory mechanism and selective advantage for diel vertical

migration in juvenile sockeye salmon, *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 47: 1796–1802.

LOVE, R.H. 1971. Dorsal-aspect target strength of an individual fish. J. Acoust. Soc. Am. 49: 816–823.

MCNAMARA, J.M., AND A.I. HOUSTON. 1986. The common currency for behavioral decisions. Am. Nat. 127: 358–378.

MUNK, P., T. KIØRBOE, AND V. CHRISTENSEN. 1989. Vertical migrations of herring, *Clupea harengus*, larvae in relation to light and prey distribution. Environ. Biol. Fishes 26: 87–96.

NARVER, D.W. 1970. Diel vertical movements and feeding of underyearling sockeye salmon and the limnetic zooplankton in Babine Lake, British Columbia. J. Fish. Res. Board Can. 27: 281–316.

NEILL, W.H., AND J.J. MAGNUSON. 1974. Distributional ecology and behavioral thermoregulation of fishes in relation to heated effluent from a power plant at Lake Monona, Wisconsin. Trans. Am. Fish. Soc. 103: 663–710.

NORTHCOTE, T.G., AND H.W. LORZ. 1966. Seasonal and diel changes in food of adult kokanee (*Oncorhynchus nerka*) in Nicola Lake, British Columbia. J. Fish. Res. Board Can. 23: 1259-1263.

NORTHCOTE, T.G., H.W. LORZ, AND J.C. MACLEOD. 1964. Studies on diel vertical movement of fishes in a British Columbia lake. Verh. Int. Ver. Limnol. 15: 940–946.

OLLA, B.L., A.L. STUDHOLME, AND A.J. BEJDA. 1985. Behavior of juvenile bluefish *Pomatomus saltatrix* in vertical thermal gradients: influence of season, temperature acclimation and food. Mar. Ecol. Prog. Ser. 23: 165–177.

PEARCY, W., T. NISHIYAMA, T. FUJII, AND K. MASUDA. 1984. Diel variations in the feeding habits of Pacific salmon caught in gill nets during a 24-hour period in the Gulf of Alaska. Fish. Bull. 82: 391–399.

RICHMAN, S. 1958. The transformation of energy by *Daphnia pulex*. Ecol. Monogr. 28: 273–291.

SCHNEIDERVIN, R.W., AND W.A. HUBERT. 1987. Diet overlap among zooplanktophagous fishes in Flaming Gorge Reservoir, Wyoming–Utah. N. Am. J. Fish. Manage. 7: 379–385.

SEABURG, K.G. 1957. A stomach sampler for live fish. Prog. Fish-Cult. 19: 137–139.

STEWART, D.J., J.F. KITCHELL, AND L.B. CROWDER. 1981. Forage fishes and their salmonid predators in Lake Michigan. Trans. Am. Fish. Soc. 110: 751–763.

SWIFT, M.C. 1976. Energetics of vertical migration in *Chaoborus trivittatus* larvae. Ecology 57: 900–914.

THORNE, R.E. 1983. Hydroacoustics, p. 239–259. *In* L.A. Nielsen and D.L. Johnson [ed.] Fisheries techniques. American Fisheries Society, Bethesda, Md.

WELLER, D.E., D.J. ANDERSON, D.L. DEANGELIS, AND C.C. COUTANT. 1984. Rates of heat exchange in largemouth bass: experiment and model. Physiol. Zool. 57: 413–427.

WURTSBAUGH, W.A., AND D. NEVERMAN. 1988. Post-feeding thermotaxis and daily vertical migration in a larval fish. Nature (Lond.) 333: 846–848.

# Recycling of Elements Transported Upstream by Runs of Pacific Salmon: II. $\delta^{15}N$ and $\delta^{13}C$ Evidence in the Kvichak River Watershed, Bristol Bay, Southwestern Alaska[1]

Thomas C. Kline, Jr., John J. Goering, Ole A. Mathisen, and Patrick H. Poe

*School of Fisheries and Ocean Science, University of Alaska Fairbanks, AK 99775-1080, USA*

and Patrick L. Parker and Richard S. Scalan

*Marine Science Institute, University of Texas at Austin, Port Aransas, TX 78373, USA*

Kline, T.C. Jr., J.J. Goering, O.A. Mathisen, P.H. Poe, P.L. Parker, and R.S. Scalan. 1993. Recycling of elements transported upstream by runs of Pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska. Can. J. Fish. Aquat. Sci. 50: 2350–2365.

Biota $\delta^{15}N$ and $\delta^{13}C$ values (deviations from recognized isotope standards) from Iliamna Lake (a major anadromous sockeye salmon (*Oncorhynchus nerka*) nursery lake supporting peak-year runs >10 million) and several other anadromous-salmon-free lakes in the Kvichak River watershed, Bristol Bay, southwestern Alaska, were compared to determine the significance of marine-derived nitrogen (MDN) delivered by returning adult salmon. Biota In Iliamna Lake had higher $\delta^{15}N$ compared with control lakes, verifying a mixing model correlating $\delta^{15}N$ with MDN. Periphyton $\delta^{15}N$ values reflected localized input from populations of spawning salmon. Juvenile sockeye MDN varied in response to escapement size, suggesting the importance of large escapements (>10 million) for maintaining a predominantly MDN lacustrine N pool. Other resident fishes showed shifts in $\delta^{15}N$ between years of high and low escapement. The dual-isotope approach, using $\delta^{15}N$ and $\delta^{13}C$ together, suggested that fish production is primarily dependent on limnetic primary and secondary production. The dual-isotope approach indicated that the coast range sculpin (*Cottus aleuticus*) was the only fish with an appreciable dietary component consisting of salmon eggs or emergent fry.

Les teneurs en $\delta^{15}N$ et $\delta^{13}C$ (écarts des étalons d'isotope reconnus) du biote du lac Iliamna (lac pépinière important pour le saumon rouge anadrome (*Oncorhynchus nerka*) qui connaît des remontes maximales de >10 millions) et plusieurs autres lacs ne contenant pas de saumons anadromes dans le bassin hydrographique de la rivière Kvichak (baie Bristol), dans le sud-ouest de l'Alaska, ont été comparées afin d'établir l'importance de l'azote d'origine marine apporté par des saumons adultes au moment de la remonte. Les teneurs en $\delta^{15}N$ du biote du lac Iliamna étaient plus élevées que celle de lacs témoins ce qui vérifie un modèle de brassage établissant une corrélation entre la teneur en $\delta^{15}N$ et l'azote d'origine marine. Les teneurs en $\delta^{15}N$ du périphyton reflétaient un apport localisé de populations de saumons reproducteurs. La teneur en azote d'origine marine chez les juvéniles de saumon rouge variait en fonction de la taille de l'échappée ce qui semble indiquer l'importance de grandes échappées (>10 millions) pour maintenir une réserve d'azote d'origine marin dans un lac. Les teneurs en $\delta^{15}N$ chez d'autres poissons résidants présentaient des variations entre les années à échappée élevé et faible. Le recours à ces deux isotopes, $\delta^{15}N$ et $\delta^{13}C$, semblait indiquer que la production de poisson dépend surtout de la production limnétique primaire et secondaire. L'approche par ces deux isotopes a montré que le chabot côtier (*Cottus aleuticus*) était le seul poisson dont le régime alimentaire comprenait une quantité appréciable d'oeufs de saumons ou d'alevins émergents.

*Received May 21, 1991*
*Accepted May 31, 1993*
*(JB045)*

*Reçu le 21 mai 1991*
*Accepté le 31 mai 1993*

Returning semelparous anadromous Pacific salmon, (*Oncorhynchus* spp.) have been shown by the measurement of the natural abundance of stable isotopes technique to be a significant nitrogen (N) source for Sashin Creek, a rapidly flushing southeastern Alaska stream (Kline et al. 1990). Ecological investigations based on the stable isotope biogeochemistry of biophilic elements hinge on the presence of an isotopic disparity between potential sources that can be detected at higher trophic levels (Wada and Hattori 1991). Biogenic N from returning anadromous Pacific salmon, virtually 100% marine in origin, has been shown to be isotopically distinguishable from terrestrial and freshwater N (most likely derived from fixation of atmospheric $N_2$) in Sashin Creek (Kline et al. 1990). Biota in the section of Sashin Creek available to spawners was found to be enriched with the heavy N isotope, $^{15}N$, when compared with biota found in the section that was upstream of a 30-m waterfall (a barrier to anadromous salmon migration). The marine N isotopic signature was conserved through the process of decomposition and remineralization into Sashin Creek food webs. This was, in part, due to the large difference in $^{15}N/^{14}N$ in returning salmon compared with that in the terrestrial environment. Because environmental conditions that could lead to $^{15}N$ enrichment by ammonia volatilization (high $NH_4^+$ and high pH)

[1]Contribution No. 973, Institute of Marine Science, University of Alaska Fairbanks, Fairbanks, AK 99775-1080, USA.

or denitrification (low $O_2$) are toxic to salmonid fishes, these processes are unlikely to occur where large populations of juvenile salmonids rear; hence, elevated $^{15}N$ can be ascribed to marine-derived N (MDN) input. The Sashin Creek study established the stable isotope abundance technique as a tracer of MDN in lotic Pacific salmon freshwater systems. This concept is extended in this study to a lentic system, Iliamna Lake, the primary sockeye (*Oncorhynchus nerka*) nursery lake in the Kvichak River system, where it has been previously established that salmon can be a major source of phosphorus (P) (Donaldson 1967).

It has been suggested that returning salmon can be a significant nutrient source to sockeye salmon nursery lakes (Juday et al. 1932; Barnaby 1944; Donaldson 1967; Krohkin 1967; Mathisen 1972; Koenings and Burkett 1987a) that are typically oligotrophic (Burgner et al. 1969; Hyatt and Stockner 1985). Nutrient release from fishes through excretion and decomposition has been identified as a top-down effect of fish on freshwater ecology (Northcote 1988). In the case of anadromous Pacific salmon, the terminal return migration can be viewed as an upstream vector of allochthonous nutrients from the marine environment. In this study, the use of variation in natural abundance of $^{15}N/^{14}N$ is applied to Iliamna Lake in the Kvichak River watershed, a major Alaskan sockeye-salmon-producing lake. Part of this study was similar to our Sashin Creek study in that control sites free of anadromous salmon were compared with sites within a spawning system. However, temporal and spatial variation in spawning density, longer residence time of salmon adults and carcasses, and the longer flushing time of the system were expected to add complexity. Of special significance was that the period of sampling, 1985–87, corresponded to a decline in the number of salmon returning to spawn in the Kvichak watershed. This made it possible to test the hypothesis that variation in escapement could result in significant changes in the nutrient budget important for the growth and survival of juvenile salmon.

## The Kvichak System

The Kvichak River watershed has been the largest producer of sockeye salmon to the Bristol Bay, Alaska, sockeye fishery. Maturing sockeye salmon ascend the Kvichak River in late June – early July to spawn in Iliamna Lake and its tributaries. The annual escapement (enumerated by the Alaska Department of Fish and Game Commercial Fisheries Division and previous fisheries research agencies at a counting site on the Kvichak River just downstream from Iliamna Lake) has ranged from 225 000 to 24 million since World War II. The number of salmon returning to spawn has historically cycled with a 4- or 5-year period in the Kvichak system (Eggers and Rogers 1987). Several hypotheses have been developed to explain the occurrence of this so-called cyclic dominance in certain anadromous salmon systems (Foerster 1968; Mathisen 1972; Collie and Walters 1987; Eggers and Rogers 1987). Mathisen (1972) suggested that biogenic nutrient feedback from large escapements could play a role in maintaining the peak cycle years in the Kvichak system. Conversely the small escapements in off-years could not sustain or build up the off-year runs. Through simulation modeling, Eggers and Rogers (1987) showed that sockeye population cycling is intrinsic in the Kvichak system. Furthermore, they found that fishing is depensatory, amplifying the cycle and creating the strong peaks. Thus, one objective of this study was to determine whether a response, in terms of MDN related to the size of escapement, could be detected in juvenile salmon. If larger escapements result in a proportional increase of MDN in juvenile sockeye compared with smaller escapements, then it could be argued that other N sources are of less importance for large juvenile sockeye populations. By extension, the productivity of the ecosystem could be seen as being density dependent with respect to sockeye escapement. Thus, a system's sockeye production may be determined as much as by the number of decomposing carcasses as the number of eggs deposited into redds by the spawning adults because both are necessary for growth and survival of the progeny.

TABLE 1. Estimations of the N pool associated with the annual spawning migration to Iliamna Lake (escapement data from the Alaska Department of Fish and Game, King Salmon, Alaska) based on N composition of returning sockeye (Mathisen et al. 1988) and the dissolved N pool in the lake (from Poe 1980).

| Datum | Number of salmon ($10^6$) | N pool ($10^9$ g) |
|---|---|---|
| 1983 | 3.6 | 0.3 |
| 1984 | 10.6 | 0.8 |
| 1985 | 7.2 | 0.5 |
| 1986 | 1.2 | 0.1 |
| 1987 | 6.1 | 0.5 |
| 1971–80 mean | 6.2 | 0.5 |
| 1981–90 mean | 5.1 | 0.4 |
| N dissolved in Lake | | 4.8 |
| Annual N flushed | | 0.7 |

### N Mass Balance in Iliamna Lake

The N pool associated with the annual spawning migration is comparable in size with the dissolved N pool lost by flushing in Iliamna Lake, suggesting the importance of adult salmon N to the N budget (Table 1). Mathisen et al. (1988) estimated that each returning mature sockeye transports 73.2 g of N at point of entry into Iliamna lake. A relatively small number of salmon returning to spawn, e.g., 1 million, would result in an input of $7.32 \times 10^7$ g of N. In comparison, a large number, e.g., 15 million, would bring in $1.1 \times 10^9$ g of N. These values are comparable with the mean of the estimated total dissolved N pool (as $NO_3^- + NO_2^-$) in Iliamna Lake of $4.8 \times 10^9$ g of N (range $2.4 \times 10^9$ to $9.7 \times 10^9$ g), (Poe 1980). He included one estimate of the dissolved N pool in winter when most of the dissolved N should have been remineralized to $NO_3^-$. The winter estimate made in March 1976 was $9.7 \times 10^9$ g of N ($NO_3^- + NO_2^-$) based on the nutrient profile taken at the upper end of Iliamna Lake (Poe 1980). Assuming that the concentration of dissolved N in the lake outflow equals the mean for the lake, then the annual N loss through flushing, based on the 7.2-yr flushing time, is $0.7 \times 10^9$ g using the mean of Poe's (1980) dissolved N estimates, or $1.3 \times 10^9$ g using the winter 1976 estimate of the total dissolved N pool. The N input in a peak-year (e.g. 1984, Table 1) spawning migration is equivalent to 50–100% of the estimated annual N pool loss by flushing. This is in agreement with Donaldson's (1967) estimate that a peak-year input of marine P supplies >50% of the lake P pool. The emigration of sockeye smolts also removes N from the lake. However, the smolt biomass is about 1–2 orders of magnitude smaller than the adult immigration biomass, so the smolt component is small in terms of the N budget in a first-order analysis. However, they could be relatively important in small-escapement years if many smolts are emigrating. In these cases, smolt biomass could be equivalent to adult biomass. Thus, this nutrient loss would be barely maintained by new MDN input.

TABLE 2. Physical characteristics of Iliamna Lake, Alaska (from Poe 1980).

| System characteristic | Dimension |
|---|---|
| Surface area | 2622 $km^2$ |
| Catchment basin[a] | 20720 $km^2$ |
| Volume | 117 $km^3$ |
| Maximum depth | 393 m |
| Mean depth | 44.1 m |
| Maximum length | 125 km |
| Maximum width | 65 km |

[a] Includes Lake Clark with a catchment basin of 9583 $km^2$.

Estimations of the MDN input during the course of this study, averages for the two most recent decades, and dissolved N pool in Table 1 suggest that salmon N input can be the equivalent of the N pool loss through flushing and thus should be important in maintaining a steady-state nutrient pool in the lake. The above analysis, basically a mass-balance approach, only provides indirect evidence that returning salmon deliver significant quantities of nutrients important to freshwater ecosystems. The advantage of the natural stable isotope abundance technique is that the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ signatures can be traced directly in ecosystem components, i.e., the biota, providing direct and quantitative evidence of the flow of nutrients from different sources. This paper is a continuation of the development of this technique in salmonid freshwater habitats that was initiated in Sashin Creek (Kline et al. 1990).

### Timing of Events Related to Iliamna Lake N Pools

Because migrating salmon stop feeding prior to reentry into freshwater, all subsequent energy and nutritional requirements have to be internally derived from elements previously acquired from the marine environment. Elements lost through excretion, spawning, and eventually decomposition are thus marine derived.

Spawning commences in early August and continues until October. Although carcasses do not appear until mid-August, excretion by adult salmon during the final part of gametogenesis, in freshwater, is a potential source of dissolved nutrients (Mathisen et al. 1988). Comparison of the elemental composition of adults sampled upon entry into freshwater with newly spent salmon showed that a significant quantity (30%) of N is lost as excretion and gametes (Mathisen et al. 1988). Spawning occurs in small streams, rivers, and springs that drain into Iliamna Lake and several tributary lakes. Spawning also occurs at many lake beach sites. An ROV (remotely operated vehicle) survey of several Iliamna Lake beach spawning sites in early July, 1988 revealed the presence of incompletely decomposed sockeye carcasses at 20–50 m depth, suggesting the potential for nutrient input for a period of more than 8 mo beyond the termination of spawning.

Emergence of fry from the gravel occurs in the spring. Initial feeding occurs in the littoral zone of the nursery lakes. By August, all fry are in the limnetic zone where they forage on zooplankton until migrating out to sea as 1- or 2-yr-old smolts. Limnetic feeding in Iliamna Lake by sockeye fry (young-of-the-year) and yearlings (age 1+) is selective on the cladoceran *Bosmina* and the copepod *Cyclops*, respectively (Hoag 1972).

### Characteristics of Iliamna Lake

Iliamna Lake (59°N, 155°W) is the largest freshwater body in Alaska (Table 2). The Iliamna Lake environment includes areas of both coastal and continental climates and is characterized by strong winds (Williamson and Peyton 1962). Summers are typically wet and winters are dry. Lake level fluctuates, with the peak occurring in September and declining to a minimum in April–May (Donaldson 1967; Poe 1980). Most of the flushing takes place during the summer and fall when ~20% of the lake volume drains out the Kvichak River during high water (Donaldson 1967). Strong winds maintain a deep mixed layer, with the result that the lake is poorly stratified. Iliamna Lake is usually ice covered from January to May (Poe 1980). A peak in the nutrient concentration profile just below the ice in March suggests a possible input from leaching of previous periphyton blooms exposed during low water and the effect of subice stratification (P.H. Poe and W.S. Reeburgh, unpublished data). Iliamna Lake is classified as oligotophic (Burgner et al. 1969). Limnetic summer primary productivity has been estimated as 142–213 mg $C \cdot m^{-2} \cdot d^{-1}$ (Low 1972). The human population in the area is <1000, so anthropogenic input of N is small (much of it is salmon derived, as salmon play a major part in the local nutrition).

The Kvichak system, in addition to being an important nursery for sockeye salmon, supports ~25 resident fish species (R. Russell, Alaska Department of Fish and Game, King Salmon, Alaska, personal communication). An aspect of this project was the comparison of $^{15}N/^{14}N$ in food webs of systems without anadromous salmon with those impacted by anadromous salmon (Kline et al. 1990). To this end, comparisons were made between isotopic signatures of fishes of the same species in both salmon and nonsalmon lakes. The following species in lakes of the Kvichak watershed were compared: coastrange sculpin (CRS) (*Cottus aleuticus*), threespine stickleback (3SP) (*Gasterosteus aculeatus*), ninespine stickleback (9SP) (*Pungitius pungitius*), rainbow trout (RBT) (*Oncorhynchus mykiss*), and Dolly Varden (DV) (*Salvelinus malma*). One Arctic Char (*Salvelinus alpinus*) sampled at Kakhonak Lake was included with the DV data. Identification of *Salvelinus* was based on gillraker count (McPhail and Lindsey 1970).

## Materials and Methods

### Sites

Sampling in the Kvichak River watershed was concentrated near the major sockeye spawning grounds in the eastern end of Iliamna Lake and in nearby nonsalmon control lakes. Iliamna Lake sampling was done at established stations (Donaldson 1967; Mathisen 1972; Poe 1980) and at additional selected sites (Fig. 1).

Five established littoral zone sampling stations (Mathisen 1972; Poe 1980) were supplemented by seven additional stations and sampled during most visits. Littoral stations were classified in relation to proximity to, and density of, beach spawning. Three stations located where large colonies of sockeye spawn (thousands of spawners) were classified as high density (HDN). Two stations adjacent to medium-sized (hundreds of spawners) or small colonies of spawners (<100 spawners), depending on year, were classified as low density (LDN). Four other stations >1 km away from spawning areas, but located where carcasses could drift in, were classified as drift sites (DRF). A spring-fed pond, Knutson Spring (KSP), used as a spawning site was sampled because of abundant blue-green algae. Another station, located

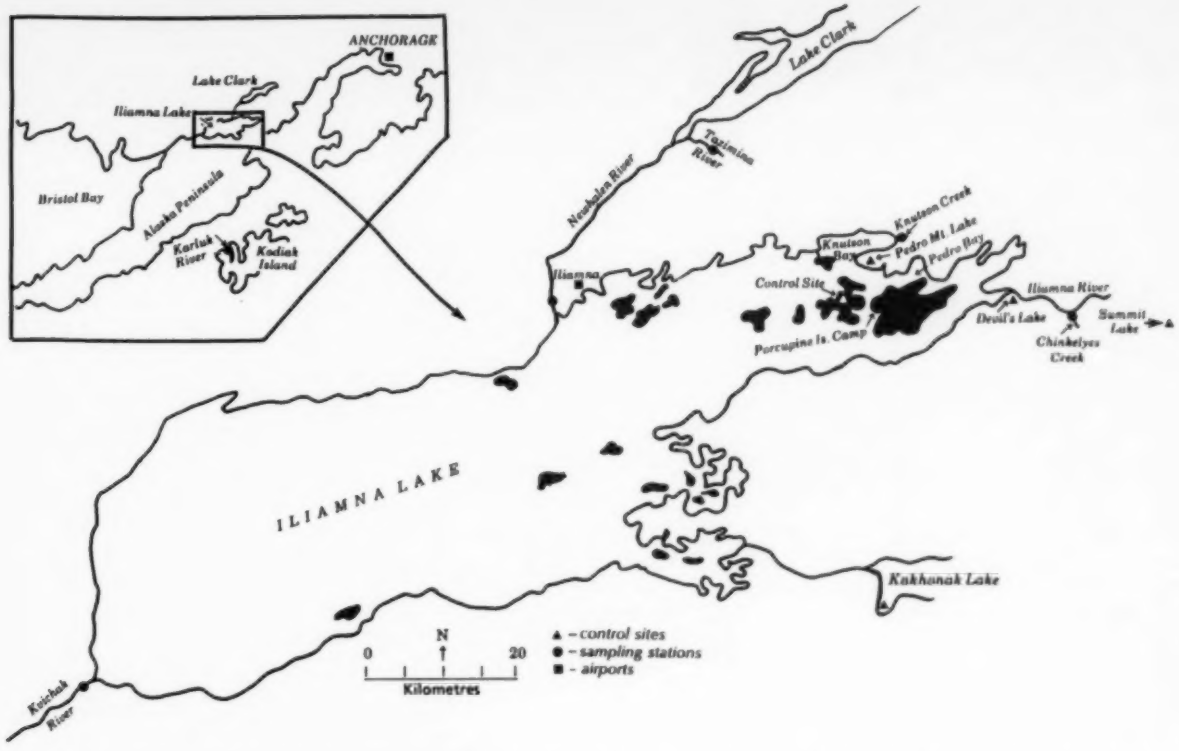



FIG. 1. Map of Iliamna Lake area showing geographical locations mentioned in text. Sites where adult salmon were sampled are indicated by circles. Knutson Spring is located adjacent to the mouth of Knutson Creek. Control sites are indicated by triangles. Towns with airports are indicated by squares. Islands within Iliamna Lake are black for clarification.

within the intricate channels of an island in the middle of Iliamna Lake, was classified as a control site within Iliamna Lake (Fig. 1). This control site, also used as a control in a previous periphyton study (Mathisen 1972), supplemented the non-anadromous salmon lakes that collectively were classified as controls (CTL).

Limnetic sampling was conducted at stations in Iliamna Lake established since the 1960s by the University of Washington Fisheries Research Institute. These included plankton tows at stations that are representative of the middle and upper portions of Iliamna Lake where many of the juvenile salmon rear, and tow-net sampling on established cruise tracks running through Knutson and Pedro bays in the northeastern part of the lake for limnetic fish (primarily juvenile sockeye salmon). Additional opportunistic sampling was also done at numerous locations in the watershed.

A small (~40 ha) salmon-free lake, located near the mouth of the Iliamna River, known as "Devil's Lake" by the residents of Pedro Bay, was established as a control site for repeated sampling throughout the course of this study. Other control lakes that had limited accessibility, Kakhonak Lake, Pedro Mountain Lake, and Summit Lake (Fig. 1), were sampled once each to extend the control lake $\delta^{15}N$ baseline. These control sites, although part of the Kvichak watershed, have no anadromous salmonids because of impassable waterfalls (Demory et al. 1964). Littoral zone samples collected in these lakes were compared with samples from the Iliamna Lake littoral stations classified as HDN, LDN, and DRF.

## Sampling Schedule

RETURNS (Recycling of Elements Transported Upstream by Runs of Pacific Salmon) project sampling was conducted over a 2-yr period, 1985–86. A few samples were collected during a 1983–84 pilot study. A single visit in August of 1987 was made to sample a few Iliamna Lake sites using scuba to obtain subsurface littoral biota and to sample at Kakhonak Lake for fishes, periphyton, and plankton. Personnel of the Commercial Fisheries Division, Alaska Department of Fish and Game, collected smolts during their annual emigration studies at the outlet of Iliamna Lake. The multiyear sampling program at Iliamna Lake allowed for comparison of MDN among years that exhibited a large range in escapement (10.2, 7.2, and 1.2 million in 1984, 1985, and 1986, respectively). A visit made to Iliamna Lake in early July 1988 to conduct an ROV survey provided an opportunity to collect a few additional samples including adults from the Newhalen River. In 1988, we had the opportunity to sample adults from another system (Karluk River on Kodiak Island, see Fig. 1).

## Field and Laboratory Methods

Collection and sample preservation methods (desiccation over silica gel or freezing at −20 °C) have been described by Kline et al. (1990). Monofilament gill nets, tow nets, baited minnow traps, and angling were used to sample resident fishes. Vertical and diagonal tows with 73-, 130- and 223-μm-mesh, 0.5-m-diameter nets were used to collect plankton. A slurp gun was used for sampling while scuba diving or snorkeling at littoral sites.

Migrating adult sockeye were seined from the Kvichak River (Mathisen et al. 1988). Fully mature (unspawned) and spawned-out salmon were collected in beach seines at spawning sites (Mathisen et al. 1988). Adult sockeye salmon samples were prepared in one of three ways for analyses. (1) Whole salmon homogenized for chemical analyses (Mathisen et al. 1988) were shipped frozen to Fairbanks and then freeze-dried like other samples (see above). (2) Individual carcasses were dissected in the field laboratory to remove selected tissues and organs. These small tissue and organ samples (~1 g wet weight) were frozen in the field prior to shipment to Fairbanks and treated like other samples. (3) Migrating adults from the Newhalen River in the Kvichak system and from the Karluk River on Kodiak Island were sampled to compare isotopic variation between and among sockeye salmon of these two runs. Based on conclusions drawn from the tissue samples, two specific tissues, muscle and whole gonad, were dissected and frozen as in (2) in the group (3) samples. The muscle tissue was dissected out of a transverse section located just behind the head. The group (3) samples consisted of all the sockeye salmon netted or trapped at the site at the time of sampling. The salmon collected for whole-fish analysis, group (1), were stratified by sex and ocean-age on site.

Laboratory preparation and mass spectrometry were performed as described by Kline et al. (1990). Selected samples were also analyzed at the University of Texas (Austin) Marine Science Institute, Port Aransas, Tex., using either a VG micromass 602E or Nuclide 6-60 RMS-26 stable isotope ratio mass spectrometer with similar preparation methods. Stable isotope ratios are reported relative to international standards (air for N and Pee Dee Belemnite (PDB) limestone for C) in standard delta notation:

$$(1)\quad \delta^{15}\mathrm{N} \text{ or } \delta^{13}\mathrm{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \text{ per mil}$$

where $R = {}^{15}\mathrm{N}/{}^{14}\mathrm{N}$ or ${}^{13}\mathrm{C}/{}^{12}\mathrm{C}$ (after Craig 1957). The isotope standards have delta values of 0 by definition, i.e., $\delta^{15}\mathrm{N} = 0$ for atmospheric $N_2$. Naturally occurring $\delta^{15}\mathrm{N}$ and $\delta^{13}\mathrm{C}$ values observed in biota range from ~0 to ~+20 and from ~0 to ~−50, respectively. The negative $\delta^{13}\mathrm{C}$ values reflect the relative enrichment of $^{13}\mathrm{C}$ in limestone compared with biota.

## Data Analysis

### *Estimating MDN*

The $\delta^{15}\mathrm{N}$ values of biota are dependent on two factors: (1) the $\delta^{15}\mathrm{N}$ of the N source(s) and (2) the trophic level of the organism in question. These factors were combined in a mixing model to estimate MDN from $\delta^{15}\mathrm{N}$ values (Kline et al. 1990) which is expressed mathematically by equation (2):

$$(2)\quad \%\mathrm{MDN} = \frac{\mathrm{OBS} - \mathrm{TEM}_{\mathrm{TL}}}{\mathrm{MEM}_{\mathrm{TL}} - \mathrm{TEM}_{\mathrm{TL}}} \times 100\%$$

where OBS is the observed $\delta^{15}\mathrm{N}$ value, TEM is the $\delta^{15}\mathrm{N}$ terrestrial end member (the isotope signature of the terrestrial value in the mixing model), MEM is the corresponding $\delta^{15}\mathrm{N}$ marine end member (Kline et al. 1990), and TL is the trophic level. Primary producers are defined as TL = 1, and consumers are TL ≥ 2.

In the model, $\delta^{15}\mathrm{N}$ can lie between minimal and maximal values for a given TL corresponding to 0 and 100% MDN, i.e., the TEM and MEM. Thus, for a given TL the $\delta^{15}\mathrm{N}$ could range from the terrestrial end member value, $\mathrm{TEM}_{\mathrm{TL}}$ to the marine end member value, $\mathrm{MEM}_{\mathrm{TL}}$. The a priori N sources for salmon freshwater nurseries are atmospheric $N_2$ with a $\delta^{15}\mathrm{N} = 0$ by definition and salmon N with a $\delta^{15}\mathrm{N} = +11.2 \pm 1$ (Mathisen et al. 1988). The salmon-free control value of primary producers or $\mathrm{TEM}_1$ was based on $\delta^{15}\mathrm{N}$ values for benthic algae (periphyton) collected at sites free of anadromous salmon. A $\mathrm{TEM}_1$ value of 0 was thus based on empirical data (Kline et al. 1990). The $\mathrm{MEM}_1$ value required the assumption that the localized periphyton blooms occurring at spawning sites were caused exclusively by the input of salmon-derived nutrients. In comparison, such blooms were not found in control lakes. This assumption was tested by sampling periphyton from separate spawning sites. An $\mathrm{MEM}_1$ value of +7.0 per mil was initially based on periphyton growing at sites in a stream with a high concentration of salmon carcasses (Kline et al. 1990). One purpose of the control versus nursery lake comparisons of periphyton $\delta^{15}\mathrm{N}$ was the verification of these $\mathrm{TEM}_1$ and $\mathrm{MEM}_1$ values in a lentic system, as they were prerequisites for interpretation of data on higher TL biota $\delta^{15}\mathrm{N}$ using the mixing model, equation (2).

Use of the primary producers (TL = 1) end members of TEM = 0 and MEM = +7.0 (Kline et al. 1990) in this study was based on the $\delta^{15}\mathrm{N}$ values of periphyton in Iliamna Lake and the various control lakes. Estimation of higher TL end members was based on the well-established $\delta^{15}\mathrm{N}$ trophic enrichment, $\epsilon = 3.4$ (Minagawa and Wada 1984), where $\epsilon$ is the trophic enrichment factor. Thus the $\mathrm{TEM}_2$ and $\mathrm{MEM}_2$ (herbivores) were +3.4 and +10.4, respectively. Similarly the $\mathrm{TEM}_3$ and $\mathrm{MEM}_3$ (carnivores) were +6.8 and +13.8, respectively, and $\mathrm{TEM}_4$ and $\mathrm{MEM}_4$ (secondary carnivores) were +10.2 and 17.2, respectively. Mixing lines for integer TL generated by connecting the end members listed above can be shown graphically (Kline et al. 1990). One aspect of the $\delta^{15}\mathrm{N}$ mixing model is the constant slope or constant difference in end members, e.g., the difference between $\mathrm{TEM}_1$ and $\mathrm{MEM}_1$ is 7; thus, $\mathrm{MEM}_{\mathrm{TL}} - \mathrm{TEM}_{\mathrm{TL}} = 7$. The slope of 7 was derived using periphyton $\delta^{15}\mathrm{N}$ values because of their point-source response in $\delta^{15}\mathrm{N}$ to carcass abundance (Kline et al. 1990). Thus, equation (2) reduces to

$$(3)\quad \%\ \mathrm{MDN} = \frac{\mathrm{OBS} - \mathrm{TEM}_{\mathrm{TL}}}{7} \times 100\%$$

where $\mathrm{TEM}_{\mathrm{TL}}$ is the $\delta^{15}\mathrm{N}$ value from the control system. Equation (3) was used to estimate MDN of Iliamna Lake biota by comparison with values obtained for the same organisms in control systems.

The $\epsilon$ for $\delta^{15}\mathrm{N}$ may vary ±1.1 (Minagawa and Wada 1984); thus, the precision in estimating MDN based on estimating integer TL end members should decrease with increasing TL. Rather than assigning a priori TL to organisms and then determining their MDN in the model, it is possible to estimate TL from the $\delta^{15}\mathrm{N}$ data (Fry 1988) or to compare control and salmon systems directly. In order to make direct comparisons between controls and salmon systems, we assume that the TL for a given species in control and salmon systems is consistent. Thus, the control $\delta^{15}\mathrm{N}$ value for a given species (or defined subset, such as a certain size range) is used as the TEM in equation (3) for direct comparisons with salmon nursery systems. By assuming a consistent food chain length for a given species, accuracy is increased in estimating MDN because of similarity in the number of $\epsilon$ steps, probably making error systematic. Error when using estimated TL is likely to be proportional to TL because of the uncertainty of $\epsilon$ at each feeding step. Because of the subtraction

in equation (3) the net error due to TL in estimating MDN is ~0 if TL is constant. Verification of a consistent TL was based on the distribution of $\delta^{15}N$ data in histograms (Kline et al. 1990). Sockeye offspring do not occur in the control systems by definition (there are no landlocked sockeye or kokanee in this region (McPhail and Lindsey 1970)); therefore, a priori TL = 3 (zooplanktivores) was used to determine MDN. However, sockeye juveniles are stenometric zooplankton specialists and therefore should have less variability in their isotope chemistry. The observed variability should reflect variation in isotopic abundance due to source effect (MDN versus other sources) rather than variation in diet. Thus, sockeye juveniles are probably a better indicator of the effect of change in MDN in a system using the $\delta^{15}N$ technique than opportunistically-feeding species (probably all the other fishes) where diet is likely to change.

*Multiple-isotope food web analysis*

The multiple-isotope method can be used to elucidate the flow of matter in food webs when more than two different food sources exist, if any two sources differ in stable isotope abundance of a given element. In this study, there are three aquatic food sources for fishes. Two are autochthonous sources: benthic prodution in the littoral zone and planktonic production in the limnetic zone. Sockeye salmon carcasses (unremineralized), their eggs, and emergent fry (prior to feeding on freshwater production) are collectively an allochthonous third food source for fishes. Through consumption of carcasses, eggs, and fry, resident fishes can obtain the marine $\delta^{15}N$ signature directly. Thus, consumption of adult sockeye products had to be assessed as an alternative pathway for MDN utilization compared with remineralized MDN utilization in autochthonous food webs. Qualitative analysis of Sashin Creek fishes (Kline et al. 1990) indicated that eggs and fry were not a major component in fish diets in that system. A more intensive sampling effort in this study with several fish species in both spawning and control systems (compared with rainbow trout alone in Sashin Creek) permitted a quantitative analysis of MDN flow to fishes. The analytical protocol is graphically modeled in Fig. 2. The isotopic signature of a given sample, *D*, is a mixture of three potential sources of production and thus three food chains, distinguishable by a combination of $\delta^{13}C$ and $\delta^{15}N$. Isotopic signatures for a given sample (e.g., a length range for a given species) were estimated for each potential source based on TL and known fractionation factors (1.0 and 3.4 per TL for $\delta^{13}C$ and $\delta^{15}N$, respectively). For example, a fish consuming only food chain *A* would have a value of *A′* (the shift from *A* to *A′* reflecting the fractionation factors). Estimates of isotopic signatures for the same species-sample dependent on only *B* and *C*, *B′* and *C″*, were derived in a similar manner although with a shorter food chain and hence, a different fractionation factor for *C*. A fish feeding exclusively on eggs and fry (a single isotopic signature assumed) would be isotopically enriched by only one trophic step compared with diets dependent on algal production that require several trophic steps. The one trophic step for feeding on eggs and fry is species independent; thus, *C″*, the signature of a fish feeding on *C* (eggs and fry), is constant. Aé and Bé vary according to TL for a given fish. For the purposes of this study, it was assumed that the food chain length from *A* to *A′* and from *B* to *B′* was the same for a given fish species.

The points *A*, *A′*, B, B′, *C*, and *C″* were empirically derived. The $\delta^{13}C$ value used here for *A* was based on the mean value of periphyton collected during observed (Mathisen 1972) peak growth periods (August–October) at littoral sites classified LDN and HDN. *A′* was derived from *A* and was dependent on the TL

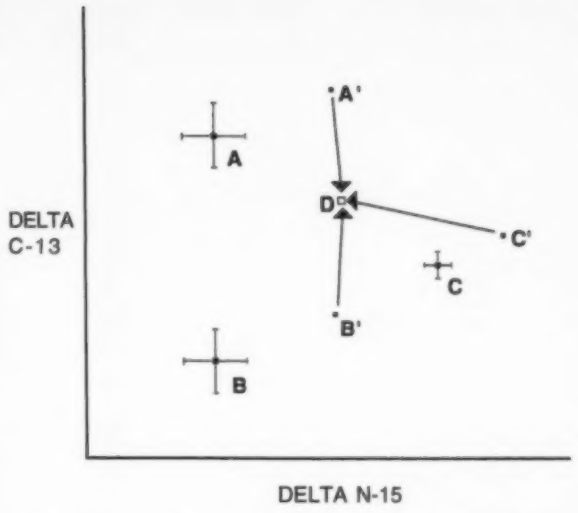



FIG. 2. Dual-isotope, three-source mixing model with variable trophic enrichments. Contribution of source *A*, *B*, or *C* was based on proximity of the observed isotopic signature *D* to end members *A′*, *B′*, and *C″*. *A′*, *B′*, and *C″* are end members corrected for trophic isotopic enrichment (e.g., a one-step trophic enrichment is 3.4 per mil for $\delta^{15}N$ and 1 per mil for $\delta^{13}C$; in the figure the two trophic steps linking *A* and *A′* and *B* and *B′* have twofold enrichments compared with the single trophic step linking *C* and *C″* ) dependent on length of food chain from sources *A*, *B*, and *C*, respectively.

of the organism in question (whose isotopic signature is indicated as D) and year because of a shift in MDN. The $\delta^{13}C$ value for *A′* equaled the sum of *A* (the mean periphyton $\delta^{13}C$) and the TL. The $\delta^{15}N$ for *A* corresponded to the primary producer $\delta^{15}N$ in the mixing model using the estimated mean % MDN for a particular year. This value was added to the product of TL and 3.4 to estimate the $\delta^{15}N$ of *A′*. The value for *B* and *B′* were similarly derived using the estimated mean $\delta^{13}C$ of phytoplankton instead of periphyton. Point *C* is the mean $\delta^{13}C$ and $\delta^{15}N$ of salmon eggs. Consumers of salmon eggs should be enriched in the heavy isotopes by exactly one trophic step. Therefore, *C″* was fixed and had a value corresponding to the mean $\delta^{13}C$ of salmon eggs + 1.0 and the mean $\delta^{15}N$ of salmon eggs + 3.4.

Estimation of the contribution of food chain *A*, *B*, or *C* in diet of *D* was based on the proximity of *D* to *A′*, *B′*, or *C″* and calculated by

$$(4)\quad \%\ X \text{ in diet} = \left(1 - \frac{(DA' + DB' + DC') - DX'}{DA' + DB' + DC'}\right) \times 100\%$$

where *X* = *A*, *B*, or *C* and *DX′* is the length of a line connecting *D* to *X′* (Fig. 2). The line lengths were determined using the isotope ratios as Cartesian points (*X*,*Y* ).

## Results

Extension of the natural stable isotope abundance methodology into a lentic system required an extensive isotopic analysis of the adult salmon and verification of isotopic contrasts in biota from lakes with and without anadromous salmon. Data on the salmon carcasses are presented first in the section titled Isotope Chemistry of Adult Sockeye Salmon, as these results form the

TABLE 3. Comparison of $\delta^{15}N$ and $\delta^{13}C$ of fresh and spawned-out whole adult sockeye salmon and individual mature adult salmon tissues ($n$ = number of fish). Fresh salmon were from the Kvichak River near the Iliamna Lake outlet; spawned-out salmon were from the Tazimina River and Chinkelyes Creek. Tissues samples were taken from mature salmon collected at Knutson Creek (Fig. 1).

| Sample | $\delta^{15}N$ | SD | $\delta^{13}C$ | SD |
|---|---|---|---|---|
| Whole salmon | | | | |
| Fresh ($n = 4$) | +11.6 | 0.3 | −22.1 | 0.9 |
| Spawned-out ($n = 16$) | +12.3 | 0.9 | −19.6 | 0.4 |
| Tissues | | | | |
| White muscle | +11.9 | 0.8 | −20.7 | 0.3 |
| Dark muscle | — | | −22.2 | 0.2 |
| Liver | +11.4 | 1.3 | −22.1 | 0.4 |
| Eggs | +12.3 | 0.1 | −23.5 | 0.3 |

basis for the rest of the study. Second, the N isotope mixing model of Kline et al. (1990) is verified for use in the lacustrine environment by illustrating the dichotomy in $\delta^{15}N$ of biota from sockeye salmon nursery lakes (Iliamna Lake) and several non-anadromous-salmon lakes within the Kvichak watershed in Biota $\delta^{15}N$ in Iliamna Lake versus the Control Sites.

The third and fourth sections in this paper contain applications of the stable isotope abundance methodology. Because of the specialized nature of the juvenile sockeye diet, a shift in $\delta^{15}N$ in response to variation in marine N input shown in the third section, MDN in Juvenile Sockeye Salmon, provides the strongest evidence that MDN can be quantified by $\delta^{15}N$. The fourth section, Dietary Sources of MDN in Iliamna Lake consumers, uses both $\delta^{15}N$ and $\delta^{13}C$, estimated annual MDN (from the third section), and TL to reconstruct the food web structure of Iliamna resident fishes.

## Isotope Chemistry of Adult Sockeye Salmon

### *Isotope chemistry of whole salmon*

The 20 homogenized whole adult sockeye salmon carcasses analyzed here for $\delta^{15}N$ and $\delta^{13}C$ were previously analyzed for chemical constituents (Mathisen et al. 1988). These samples reflected chemical changes commensurate with utilization of internal matter during maturation and spawning in freshwater because adult salmon do not feed. Four of the adult salmon were collected in June 1985 on the Kvichak River near the Iliamna Lake outlet. These four fish were bright silver in color and had only slight development of the extended jaws or kype that characterize fully mature Pacific salmon. These were classified as fresh adult salmon for comparison with spawned-out salmon. The variability in $\delta^{15}N$ of fresh adult sockeye salmon was very small (Table 3). There was greater $\delta^{13}C$ variation (Table 3), although the SD was <1 per mil. The variation was due to slight differences in both sex and ocean age (up to 1.5 per mil difference in means, Kline 1991) that could be accounted for by differences in lipid storage because of the tendency of lipids to be depleted in $^{13}C$ (DeNiro and Epstein 1977; M. Hatano, K. Takahashi, and O.A. Mathisen, unpublished data).

Carcasses of 16 spawned-out salmon from two sites (Mathisen et al. 1988) were collected in September 1985. These samples, comprising two male and two female carcasses from both the 2- and 3-ocean-age group from each site, were analyzed for $\delta^{15}N$ and $\delta^{13}C$. Differences among spawned-out carcasses were small (Kline 1991). The females from one site and the males from the other had higher $\delta^{15}N$ values (difference in means were 1.0 and 1.3, respectively) compared with the opposite sex. There was very little variation in $\delta^{13}C$.

Differences in isotopic values between fresh and spawned-out adult salmon were not unexpected. During the 2- to 3-mo residence in the Iliamna system, adults lose an estimated 30% of N and 60% of C as excrement and gametes prior to death and decomposition (Mathisen et al. 1988). Statistical analysis suggested no relationship between spawning condition (either fresh or spawned-out and $\delta^{15}N$ (all $p \geq 0.22$, ANOVA). In contrast, spawning condition was highly significant as a factor affecting $\delta^{13}C$ ($p < 0.01$, ANOVA).

Although a greater $\delta^{15}N$ variability in spawned-out salmon compared with fresh salmon (Table 3) reduced statistical significance, the higher $\delta^{15}N$ in the spawned-out salmon suggests that the initial N fraction (30% of the original N pool) lost prior to decomposition could have a slightly lower $\delta^{15}N$. An estimate of +10 for the $\delta^{15}N$ of the 30% fraction can be made on the assumption of mass balance of the +11.6 value for the imported N pool and +12.3 for the 70% fraction remaining for release during decomposition as measured in the moribund spawned-out salmon. A fluctuation in $\delta^{15}N$ from ~+10 to +12.3 of the N released into freshwater from the time of entry into Iliamna Lake (early July) to decomposition (peak from September to October) had to be considered in the interpretation of $\delta^{15}N$ values, especially for those organisms capable of showing a rapid response to shifts in $\delta^{15}N$ of the N source, i.e., periphyton (Kline et al. 1990). However, this variation is small compared with the isotopic disparity of 7 per mil in the isotope model that represents source effect (marine versus terrestrial) on the observed $\delta^{15}N$ values of biota.

### *Small tissue samples*

We established in the previous section that an isotopic shift occurs as adult sockeye salmon mature and eventually spawn in freshwater. It was necessary to study the variability in this pattern over time using large-scale field sampling. Because transporting large whole carcasses in remote areas is logistically difficult, we developed an alternative protocol whereby small subsamples of tissues could be isotopically analyzed.

Mizutani et al. (1991) stated that $\delta^{15}N$ and $\delta^{13}C$ in individual cormorant tissues are linearly correlated with diet. They found a consistent isotopic enrichment in the individual tissues in both N and C. Although no explanation was given for this relationship, they estimated that the mean $\delta^{15}N$ enrichment of individual tissues from diet was approximately +3 with a 4.0 range between different tissues. They found that the $\delta^{13}C$ enrichment between

TABLE 4. Comparisons of $\delta^{15}N$ and $\delta^{13}C$ in adult (bright, semi-mature, six fish per sex–site) sockeye salmon white muscle tissue from the Newhalen and Karluk rivers and gonad tissues from the Newhalen River.

| | Newhalen River | | | | Karluk River | |
|---|---|---|---|---|---|---|
| | Gonad | SD | Muscle | SD | Muscle | SD |
| $\delta^{15}N$ | | | | | | |
| Males | +11.8 | 0.2 | +12.0 | 0.5 | +11.0 | 0.6 |
| Females | +11.6 | 0.3 | +11.3 | 0.3 | +11.4 | 0.5 |
| $\delta^{13}C$ | | | | | | |
| Males | −19.6 | 0.7 | −21.7 | 0.9 | −21.0 | 0.6 |
| Females | −23.4 | 0.3 | −21.6 | 0.6 | −20.2 | 0.5 |

individual tissues from diet was greater than the +1 that is generally accepted for enrichment between whole animals and their diet. The $\delta^{13}C$ enrichment range was 3.1 (no weighted mean was given). Because of the lack of consistency between tissues and diet, it is thus best to analyze whole organisms if the data are being used for ecological studies. If a smaller sample is more practical, then it must be established that the subsample taken is representative of the whole organism. Mature, pre-spawned sockeye were used for the tissue sample analysis (Table 3). White muscle tissue values were pooled when several different sections were sampled from within an individual fish because the $\delta^{15}N$ and $\delta^{13}C$ data within fish were found to be consistent. For example, the isotopic variation of white muscle within a fish was small enough (<1 per mil) to allow us to use subsamples in subsequent sampling of adult salmon. The white muscle tissue of mature salmon was slightly $^{13}C$ enriched compared with whole fresh salmon but $^{13}C$ depleted relative to whole spawned-out salmon (Table 3). These data and the more negative $\delta^{13}C$ of dark muscle and eggs are in accordance with the lipid content of these tissues and the expected depletion in $^{13}C$ of lipids (DeNiro and Epstein 1977). During the maturation process the salmon utilize stored energy in the form of lipids from the white muscle (M. Hatano et al., unpublished data); thus, a change in $\delta^{13}C$ was not unexpected. The significance of these results is that the eggs and spawned-out carcass tissue have distinguishable $\delta^{13}C$ for use in food web studies where differentiation of eggs from carcasses is important.

*Adult isotopic variability*

Small inter- and intraorganismal variations in sockeye stable isotope chemistry allowed sampling from only muscle and gonads. White muscle tissue $\delta^{15}N$ was considered representative of the whole carcass (Table 3). We also sampled eggs because they are a potential food source for resident fishes. Their distinct isotope signature (Table 3) allowed us to determine whether or not they were consumed by fishes.

Differences in $\delta^{15}N$ and $\delta^{13}C$ between muscle and eggs were suggested in the small tissue sample analysis. To better estimate the isotopic variability within a synoptically sampled salmon population (to avoid incorporating isotopic shifts during maturation), comparisons of muscle and gonads (ovaries and testes) were made where data (Table 4) were most comparable with the four fresh salmon sampled in 1985 (Table 3). Multifactorial ANOVAs were performed to determine the relationship of tissue variability (muscle versus gonad) and sex on isotope chemistry of adult salmon. Sex had a significant effect on $\delta^{15}N$ ($p < 0.05$, Table 4). Sex and sex–tissue interactions had a significant effect

TABLE 5. Comparison of periphyton $\delta^{15}N$ between controls and Iliamna Lake by site type (occurrence and density of spawning). Control sites included control lakes (CTL) and Knutson Spring (KSP). Periphyton in the latter consisted of blue-green algae. Sites affected by salmon are indicated as DRF, LDN, and HDN for drift input, low spawning density, and high spawning density, respectively. MDN based on isotope mixing model with 0 and +7 as the primary producer end members. The left column includes all data (collected throughout the year) whereas the right column includes only data from the period of salmon spawning.

| | Annual | | | August–September | | |
|---|---|---|---|---|---|---|
| Site type | $\delta^{15}N$ | SD | %MDN | $\delta^{15}N$ | SD | %MDN |
| Control sites | | | | | | |
| CTL | −0.5 | 1.2 | — | +0.0 | 1.3 | — |
| KSP | +0.8 | 2.1 | — | −1.6 | 1.0 | — |
| Salmon site types | | | | | | |
| DRF | +3.9 | 3.2 | 46 | +6.3 | 3.4 | 90 |
| LDN | +3.9 | 1.6 | 46 | +3.6 | 1.8 | 51 |
| HDN | +6.1 | 3.7 | 87 | +6.6 | 4.2 | 94 |

on $\delta^{13}C$ ($p < 0.01$), with eggs and testes being, respectively, isotopically lighter and heavier than muscle (Table 4). The collective means of ovaries and testes $\delta^{13}C$ were the same as for muscle and so were insignificant when compared ($p > 0.4$). These results corroborate that the C isotopic shifts found in whole carcasses were due to gametogenesis.

Because the isotope chemistry of white muscle tissue (the bulk of the salmon carcass) was representative of whole carcasses, it was used for comparisons on a large scale. Extension of the isotopic mixing model to other systems requires that comparable end members exist (see Kline et al. 1990 for discussion on end members). This is verified here for the marine end member by comparison of the Newhalen River sample with the Karluk River (Kodiak Island) run. An opportunity to collect returning adult sockeye salmon from the Karluk River, Kodiak Island, Alaska, in addition to fish from the Newhalen River (Fig. 1) of the Kvichak system in 1988 enabled an intersystem isotopic comparison of returning adults (Table 4). The Karluk River salmon were sampled at the Alaska Department of Fish and Game weir located 1 km upstream from salt water. The chronological sequence of the Karluk sockeye spawning migration includes early-and late-run fish (Gard et al. 1987). The Karluk salmon, obtained in mid-August (1988) during the late run, were closer to spawning physiologically (more pronounced jaw, less external silvering, larger gonads), spatially (shorter migration), and temporally (earlier spawning time) than the Newhalen sample. Thus, the state of maturation was not the same in the two samples, although both populations were approaching maturation and had reared in the Gulf of Alaska for a comparable period. Nevertheless, the isotopic similarity of returning adults from two geographically distinct systems increases the potential for use of the stable isotope model with other anadromous Pacific salmon nursery systems. Two-factor (sex and system) ANOVAs were performed to test for differences in $\delta^{15}N$ and $\delta^{13}C$ in Newhalen and Karluk sockeye adult white muscle. Newhalen and Karluk male adult sockeye salmon differed in $\delta^{15}N$ by about 1 per mil whereas females were the same (sex–system interactions and system $p < 0.05$). Because the female $\delta^{15}N$ values were the same as the pooled mean, +11.4, and mean of the male $\delta^{15}N$ was +11.5, sex was an insignificant factor affecting $\delta^{15}N$ ($p = 0.6$). Newhalen and Karluk $\delta^{13}C$ values differed significantly by about

TABLE 6. Comparison of consumer time-integrated $\delta^{15}N$ between Iliamna Lake and control lakes. TL estimated on assumption of $\epsilon = +3.4$ and control primary producer $\delta^{15}N = 0$. MDN in Iliamna Lake based on assumption of same TL as controls. Fishes segregated by size when size was a statistically significant factor ($p < 0.01$, ANOVA) affecting $\delta^{15}N$. CRS = coastrange sculpin, DV = Dolly Varden, RBT = rainbow trout, 3SP = threespine stickleback, 9SP = ninespine stickleback, CFL = caddis fly larvae.

| | Controls | | Iliamna Lake | |
|---|---|---|---|---|
| Biota | $\delta^{15}N$ | TL | $\delta^{15}N$ | %MDN |
| Net plankton | +5.0 | 2.5 | +6.8 | 73[a] |
| CRS <60 mm | +6.8 | 3.0 | +11.2 | 63 |
| CR ≥60 mm | +7.7 | 3.3 | +12.5 | 69 |
| DV | +9.2 | 3.7 | +13.2 | 57 |
| RBT <100 mm | +6.7 | 3.0 | +12.8 | 88 |
| RBT ≥100mm | +9.0 | 3.6 | +13.6 | 66 |
| 3SP | +8.2[b] | 3.4 | +10.2 | 29 |
| 9SP | +6.5 | 2.9 | +10.8 | 61 |
| CFL | +1.3 | 1.4 | +8.3[c] | 86 |

[a] TL = 1.5 used to calculate MDN.
[b] Excludes Pedro Mountain Lake.
[c] Lower mode of bimodal distribution.

1 per mil (system $p < 0.01$). Sex and sex–system interactions did not affect $\delta^{13}C$ ($p \sim 0.2$). These results in combination with the muscle and gonad comparison are further evidence that $\delta^{13}C$ shifts were a result of gametogenesis, as fish of similar maturation level show no difference in muscle $\delta^{13}C$. The $\delta^{13}C$ variation in white muscle thus has potential use as a tool for quantification of the state of maturation or degree of lipid depletion in adult salmon.

## Biota $\delta^{15}N$ in Iliamna Lake versus the Control Sites

### *Periphyton*

Control site (CTL and KSP) periphyton had $\delta^{15}N$ near 0 (Table 5), consistent with Kline et al. (1990), thus justifying the continued use of $TEM_1 = 0$, the terrestrial end member in equations (2) and (3), for lake systems. The mixing model primary producer marine end member ($MEM_1$) or $\delta^{15}N$ value of a plant solely deriving its N from the salmon was assumed to be +7.0 (Kline et al. 1990). Salmon-influenced site periphyton $\delta^{15}N$ data are converted to %MDN in (Table 5) using these mixing model end member values.

%MDN estimations for periphyton growing at sites with different spawning densities ranged from ~50 to ~90% (Table 5). Low spawning density sites (LDN) had ~50% MDN with relatively small variation. High density sites (HDN) were high (~90% MDN) but with high variance among samplings. The mean values probably reflect the MDN input by averaging-out effects that cause variation in isotopic discrimination by algae and the potential variability in $\delta^{15}N$ of N released from salmon. The drift sites (DRF) varied considerably whether annual or just the spawning season was considered, reflecting the ephemeral nature of drift sites as carcasses can float in and out of the sites with vagaries in wind direction (typically east or west along axis of the lake). The $\delta^{15}N$ values thus suggest that littoral algae respond in a point-source manner to local N supply as determined by the presence of salmon carcasses. Marine N dominated (~90% MDN) as a N source at HDN spawning sites and areas where carcasses were washed up. The biogenic N signal persisted over all sampling times (virtually year-round) at HDN spawning sites (Table 5). The tendency for mean values to be ~50% MDN suggests a time-and-space integrated value for sites away from HDN spawning areas in the eastern portion of Iliamna Lake. Thus, MDN provides about half the N budget in these littoral areas.

### *Consumers*

MDN was estimated in Iliamna Lake consumers by comparing the same organisms from control lakes within the Iliamna Lake catchment basin and by analysis of TL relative to periphyton TL which was assumed to be 1. Control lake consumer TL estimates were based on $\epsilon = +3.4$ (Minagawa and Wada 1984). Trophically, the lowest consumers examined were caddis fly larvae (CFL) and zooplankton. The low $\delta^{15}N$ in control CFL (Table 6) suggests a diet of $\delta^{15}N = -2.1$ (assuming $\epsilon = +3.4$), at the low end of the control periphyton $\delta^{15}N$ (Table 5). This may indicate the presence of micro- and meiofaunal consumers (bacteria, protozoans, and small invertebrates that could not be practically separated from algae) in the periphyton samples or selection of an isotopically light diet by control CFL. Iliamna Lake CFL $\delta^{15}N$ values were strongly bimodal. The higher mode ($\delta^{15}N = +13.2$) Iliamna Lake CFL were probably carnivores and thus not comparable with control CFL. The lower mode CFL, $\delta^{15}N = +8.3$, were assumed to be mostly herbivores and more comparable with control lake CFL (Table 6).

Net plankton $\delta^{15}N$, like the periphyton, may vary spatially and temporally because of localized input of MDN from decomposing carcasses, from excretion by adult salmon (Mathisen et al. 1988), and from N cycling per se. Additionally, plankton samples were an assemblage of both phytoplankton and zooplankton in varying ratios. Phytoplankton in Iliamna Lake consists largely of chain diatoms (e.g., *Fragilaria* and *Tabellaria*) that cannot be separated from the similarly sized zooplankters *Cyclops* and *Bosmina*. Thus, the plankton samples consist of organisms from several trophic levels which leads to further $\delta^{15}N$ variability because of trophic isotopic enrichment (Wada et al. 1991). Furthermore, control lake net plankton samples had few phytoplankton and comparatively more rotifers than samples from Iliamna Lake and thus, they were not as useful for control versus Iliamna Lake comparison as either periphyton or higher trophic levels (i.e., fishes). The $\delta^{15}N$ means of each of the four control lake plankton data sets were averaged together to obtain a control grand mean $\delta^{15}N = +5.0 \pm 0.9$ (SD). Assuming that the periphyton $\delta^{15}N = 0$ applies to the phytoplankton, then the control net plankton have an effective TL = 2.5 (calculated using a +3.4 per TL enrichment). The mean $\delta^{15}N$ of Iliamna plankton samples was +6.6 ± 1.3 (SD). Based on the greater proportion of phytoplankton, the effective TL for Iliamna net plankton was probably lower than the controls. Thus, TL = 1.5 and 2.0 were used to estimate net plankton MDN = 73 and 49%, respectively. A TL = 1.5 is probably closer to the true value assuming that the plankton should resemble fishes in MDN (Table 6). Although the difference in $\delta^{15}N$ means between Iliamna Lake and controls was only 1.6, a one-way ANOVA ($p < 0.05$ ) suggested a statistically significant difference.

TL of control fishes ranged from 2.9 to 3.7 (Table 6), appropriate to a food web scheme of insect consumption and piscivory. Control threespine sticklebacks (3SP) had a broad range of $\delta^{15}N$ values (Fig. 3). Pedro Mountain Lake 3SP $\delta^{15}N$ values (mean = +11.2 ± 1.4 (SD), N = 9 fish) were enriched by +5.7 compared with Pedro Mountain Lake net plankton $\delta^{15}N = 5.5 \pm 1.0$ (SD) equivalent to an enrichment by 1.7 trophic levels. Other control lake 3SP $\delta^{15}N$ values (mean = +8.2 ± 0.8 (SD), $n = 26$ fish) were

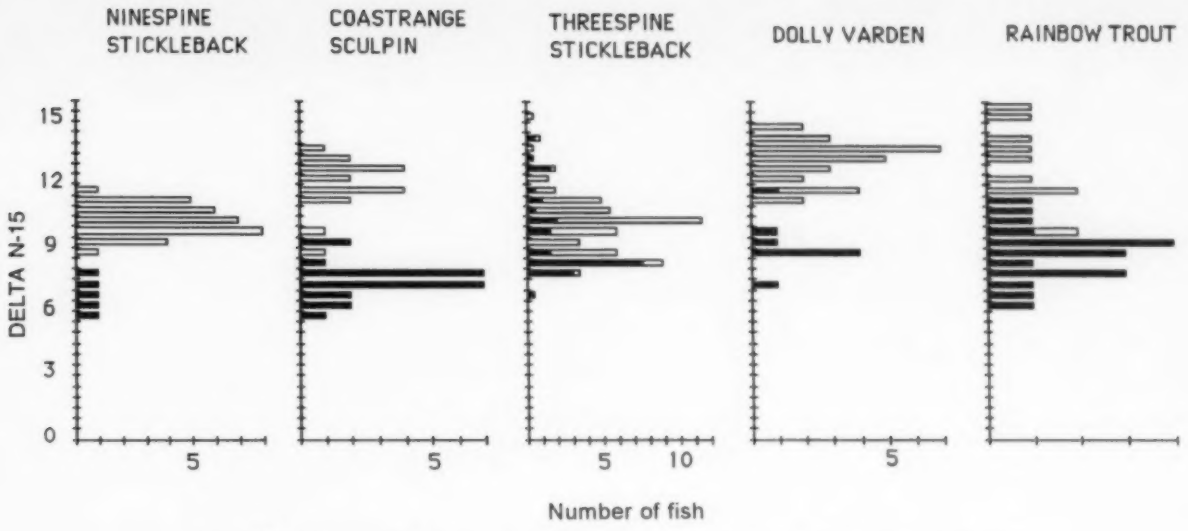



FIG. 3. $\delta^{15}N$ histograms of fishes sampled in Iliamna Lake (open bars) and control lakes (solid bars).

enriched from their net plankton ($\delta^{15}N = +5.0 \pm 0.9$) by +3.2, equivalent to 0.9 trophic level. The apparently longer food chain, as well as greater breadth in feeding niche as expressed in terms of the SD of the 3SP $\delta^{15}N$ mean, may be related to the fact that they were the only fish present in Pedro Mountain Lake (P.H. Poe, personal communication). Comparison of control lake and Iliamna Lake 3SP $\delta^{15}N$ was therefore made on 3SPs from control lakes other than Pedro Mountain Lake (Table 6). Iliamna coastrange sculpin (CRS) and rainbow trout (RBT) had large $\delta^{15}N$ ranges (Fig. 3) indicative of prey of several different TL. Fish $\delta^{15}N$ values may be dependent on size (Kline et al. 1990). Division of CRS and RBT into size classes (Table 6) was based on size frequency distribution and $\delta^{15}N$. These comparisons assumed that there was a correspondence in trophic shifts for similar sized fish in both type systems. The two estimates of MDN in CRS were similar, but in RBT were different (Table 6). The differences in mean $\delta^{15}N$ for the two size classes of CRS were similar in both control lakes and Iliamna Lake. However, the RBT intra-size-class $\delta^{15}N$ differences were 2.3 and 0.8 in control lakes and Iliamna Lake, respectively. Thus, RBT trophic structure may vary between controls and Iliamna Lake (RBT apparently occupy more than one TL in controls similar to the control section of Sashin Creek, Kline et al. 1990). If the mean $\delta^{15}N$ of Iliamna Lake RBT ($\delta^{15}N = +13.4$) is compared against either control lake size group, then estimations of MDN are 96 and 63% for TL =3.0 and 3.6, respectively. A TL = 3.6 may be more appropriate for Iliamna Lake RBT because 63% MDN is consistent with the other fishes. This assumes that all fishes should have a similar MDN component.

Dolly Varden (DV) and ninespine stickleback (9SP) $\delta^{15}N$ values were handled as unimodal due to a limited number of control samples for comparison (Fig. 3). Furthermore, the histograms of Iliamna Lake DV and 9SP have strong central tendencies. Comparison of mean $\delta^{15}N$ values suggests that these fishes are ~60% MDN.

Other than 3SP, Iliamna Lake fishes had similar estimations of MDN, 57–69%. These data suggest that returning salmon have a profound effect on the food webs supporting these fishes.

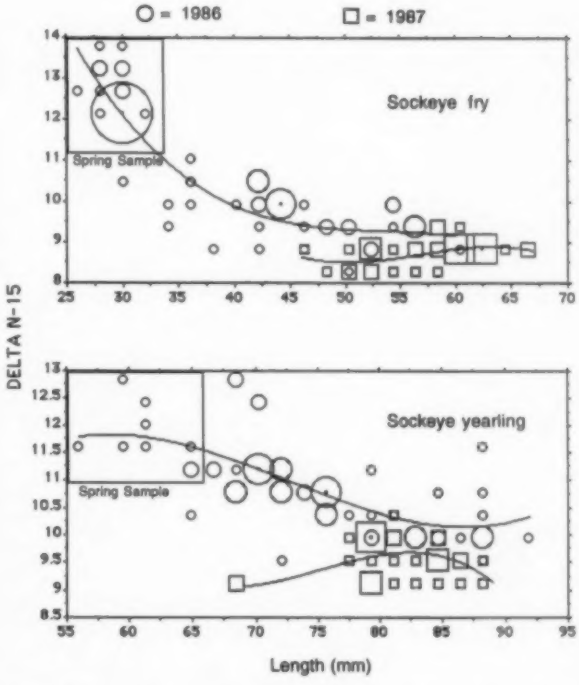



FIG. 4. $\delta^{15}N$ as a function of fork length in Iliamna Lake sockeye salmon fry and yearlings sampled in 1986 (circles) and 1987 (squares). Winter sampling of emergent fry and spring sampling of yearlings are indicated in the boxes. Other samples were collected in late August – early September. Size of symbols indicates the number of points in the size intervals. Curves were computer fitted to suggest shifts in $\delta^{15}N$ from spring to summer during growth.

## MDN in Juvenile Sockeye Salmon

Juvenile sockeye did not occur in the control lakes, making it impossible to make direct $\delta^{15}N$ comparisons as for the other fishes. MDN estimation nonetheless had to be made because of

TABLE 7. Estimated MDN of Iliamna sockeye juveniles from three cohorts (year of spawning and size of escapement given) based on $\delta^{15}N$ and TL = 3. Spring 1985 smolts include 2. smolts from a 1982 cohort.

| Brood year, brood size, and sampling period | Life history stage | $\delta^{15}N$ | SD | *n* | %MDN |
|---|---|---|---|---|---|
| 1983, 3.6 million | | | | | |
| Spring 1985 | 1. smolt | +9.1 | 1.2 | 5 | 33 |
| Spring 1986 | 2. smolt | +9.8 | 0.6 | 13 | 43 |
| 1984, 10.2 million | | | | | |
| Spring 1986 | 1. smolt | +10.0 | 0.7 | 5 | 46 |
| Spring 1986 | Yearling | +11.8 | 0.5 | 9 | 71 |
| Summer 1986 | Yearling | +10.8 | 0.7 | 52 | 57 |
| 1985, 7.2 million | | | | | |
| Summer 1986 | Fry >40 mm | +9.5 | 0.5 | 28 | 39 |
| Summer 1987 | Yearling | +9.5 | 0.4 | 30 | 39 |
| 1986, 1.2 million | | | | | |
| Summer 1987 | Fry >40 mm | +8.7 | 0.3 | 32 | 27 |

the hypothesis of nutrient feedback from anadromous adults to offspring (e.g., Mathisen 1972) which is a central theme in this investigation. The variation in run size due to the cyclic nature of the Kvichak sockeye population (Mathisen and Poe 1981) during the years of sampling provided an opportunity to examine changes in MDN in response to changes in escapement as a test of the stable isotope approach for measuring this scale of change in the N biogeochemical cycle. Sample sizes sufficient for statistically valid results were provided by the University of Washington Fisheries Research Institute during fry surveys. A complication in the data analysis was that sockeye fry have an initial inventory of N that is 100% MDN (the egg and alevin stages; feeding commences upon emergence from the gravel). It was therefore necessary to follow changes in $\delta^{15}N$ to determine a minimal fry size for use in comparisons of fry that would have lost most of their initial marine N inventory (Fig 4). Minagawa and Wada (1984) found that following the loss of initial N inventory, the mussel *Mytilus edulis* had stable $\delta^{15}N$ values. Similarly, Iliamna Lake sockeye fry have consistent $\delta^{15}N$ values at lengths >~40–45 mm (Fig. 4). The $\delta^{15}N$ of >40-mm fry from 1986 and 1987 were statistically different ($p < 0.01$, ANOVA). The 1985 fry sampled as yearlings in June 1986 were ≤65 mm (boxed area in Fig. 4). This population when sampled later that summer (August–September) had shifted to a $\delta^{15}N$ that was 1 per mil lower (Fig. 4; Table 7). Between-year comparisons of yearlings were made with >65-mm fish because of uncertain factors that could have affected the earlier sample (e.g., residual N from 1985; internal N pool shift during winter due to remobilization). The 1986 and 1987 yearlings were distinctively different ($p < 0.01$, ANOVA). Thus, both fry and yearling sockeye showed shifts in $\delta^{15}N$ in concert with a decline in escapement from the previous fall. Time course comparison of the cohorts arising from four brood years (Table 7) suggests a strong feedback from escapement to the N pool supporting juvenile salmon food webs. The shift corresponds to a majority component of the N pool being derived from salmon after >10 million escapements and a minority component after ≤7 million escapements.

## Dietary Sources of MDN in Iliamna Lake Consumers

Multiple stable isotope ratios are useful for the resolution of the relative contribution of more than two sources of production for consumers (Peterson and Howarth 1987; Kline et al. 1990). N and C isotopic signatures remain coupled during feeding processes but are decoupled during decomposition. Thus, $\delta^{13}C$ can be used as a secondary food web tracer of autochthonous production versus allochthonous marine production. Plant $\delta^{13}C$ gradients may arise in lakes because of respired C in the dissolved inorganic C (DIC) pool (Rau 1978; Quay et al. 1986; Herczeg 1987) and depletion of near-surface DIC by photosynthesis (LaZerte and Szalados 1982; Raven et al. 1982; Herczeg 1987). Limnetic production $\delta^{13}C$ values tend to be low (Rau 1980; Fry 1986; Yosioka et al. 1989). DIC depletion results in more positive (actually less negative) $\delta^{13}C$ in plants growing in shallow water, particularly in littoral areas. This dichotomy appears in Iliamna Lake where plankton $\delta^{13}C = -28.4$ (thus, fixing the $\delta^{13}C$ coordinate for point *B* in the three-point mixing model shown in Fig. 2) and periphyton (during peak growth in August–October at LDN and HDN sites) $\delta^{13}C = -10.5$ (the corresponding $\delta^{13}C$ coordinate for point A). The $\delta^{13}C$ of salmon eggs (point C) was at an intermediate value, −23.5. However, eggs and fry had very high $\delta^{15}N$ values (Table 3; Fig. 4). Thus, identification of egg and fry consumption in the three-point model is primarily resolved based on $\delta^{15}N$. Predators having a diet consisting of 100% eggs and fry would be expected to have $\delta^{15}N$ and $\delta^{13}C$ of +15.6 and −22.5, respectively, based on established $\epsilon$ (Fry and Sherr 1984; Minagawa and Wada 1984). These values, +15.6 and −22.5, form the coordinates for point C′ (Fig. 2). Such a high $\delta^{15}N$, i.e., +15.6, was not typical of any Iliamna Lake consumer (Table 6), suggesting that eggs and fry do not constitute a major portion of the diet in most consumers. It was, however, possible to estimate the relative contribution of salmon eggs and fry, littoral production, and limnetic production in the diets of Iliamna Lake consumers because of the existence of both $\delta^{13}C$ and $\delta^{15}N$ gradients. Isotopic signatures of diets derived from littoral and limnetic production, unlike direct consumption of salmon eggs and fry, depend on food chain "length" and thus required an assumption of consumer TL and primary producer $\delta^{15}N$ which fluctuated according to size of salmon N input. These isotope signatures were the derived *A′* and *B′* coordinates in Fig. 2. Because of the apparent decline in MDN through the course of the study, separate analyses were made on consumers based on year of sampling for large samples. The 3SP were also separated by the type of habitat where they were

TABLE 8. Estimated dietary source based on $\delta^{15}N$ and $\delta^{13}C$. Littoral and limnetic diet $\delta^{15}N$ end members based on 60 and 40% MDN in 1985–86 and 1987. Dietary $\delta^{13}C$ end members based on −10.5 (at sites of significant MDN, August–October) and −30.8 (based on $\epsilon = 1$ and TL (of net plankton) = 2.4) for limnetic and littoral primary producers, respectively. A diet derived from salmon eggs and emergent fry had an end member signature of +15.6 and −22.5 for $\delta^{15}N$ and $\delta^{13}C$, respectively, based on one trophic step. Diet composition based on proximity of actual values relative to three end members as shown in Fig. 2.

| | $\delta^{15}N, \delta^{13}C$ | %littoral | %limnetic | %eggs and fry |
|---|---|---|---|---|
| Threespine stickleback (*Gasterosteus aculeatus*) | | | | |
| 1986 | | | | |
| Limnetic | +11.1, −26.3 | 10 | 90 | 0 |
| Littoral | +11.6, −24.1 | 18 | 71 | 11 |
| 1987 | | | | |
| Limnetic | +9.3, −26.3 | 10 | 90 | 0 |
| Littoral | +9.9, −23.1 | 23 | 67 | 10 |
| Ninespine stickleback (*Pungitius pungitius*) | | | | |
| 1986 | +10.7, −24.4 | 22 | 78 | 0 |
| 1987 | +9.9, −24.5 | 19 | 72 | 10 |
| Coastrange sculpin (*Cottus aleuticus*) | | | | |
| <60 mm | | | | |
| 1986 | +12.1, −13.3 | 69 | 7 | 24 |
| 1987 | +9.1, −12.7 | 79 | 21 | 0 |
| ≥60 mm | | | | |
| 1986 | +12.5, −18.5 | 43 | 43 | 14 |
| 1987 | +12.3, −17.7 | 42 | 24 | 34 |
| Sockeye salmon (*Oncorhynchus nerka*) | | | | |
| Fry >40 mm | | | | |
| 1986 | +9.5, −26.0 | 14 | 86 | 0 |
| 1987 | +8.7, −26.0 | 14 | 86 | 0 |
| Yearling | | | | |
| 1986 | | | | |
| Littoral | +11.8, −27.7 | 5 | 95 | 0 |
| Limnetic | +10.8, −27.7 | 5 | 95 | 0 |
| Yearling | | | | |
| 1987 | +9.5, −26.9 | 9 | 91 | 0 |
| Rainbow trout (*Oncorhynchus mykiss*) | | | | |
| 1985–86 | +13.1, −22.2 | 25 | 72 | 4 |
| Dolly Varden (*Salvelinus malma*) | | | | |
| 1985–86 | +13.2, −18.3 | 46 | 54 | 0 |

sampled because of significantly different $\delta^{13}C$ ($p < 0.01$, ANOVA) values between samples obtained from different habitats. Samples were also separated by size classes as previously noted. The $\delta^{13}C$ coordinate was calculated by adding the TL to the $A$ and $B$ $\delta^{13}C$ values discussed above. The $\delta^{15}N$ coordinates in $A'$ and $B'$ were equal to the product of the TL and isotopic fractionation factor of 3.4 that was added to the primary producer average value for the year. An overall importance for limnetic production is suggested by the dual-isotope analysis of fishes (Table 8). Littoral C and N sources were most significant for a benthic fish, CRS. The CRS also had the strongest indication of salmon eggs and fry in their diet, as was demonstrated by Roger (1971). The relative contribution of littoral production was small in the 3SP diet although greater for those sampled in the littoral zone. The 3SP diet source did not appear to shift in response to change in MDN.

## Discussion

### Basis for the Stable Isotope Method and Potential Sources of Variation in $\delta^{15}N$ in the Absence of Salmon

Two alternative N sources for Iliamna Lake exist, adult salmon (MDN) and the atmosphere. The premise in using $\delta^{15}N$ to trace MDN in anadromous sockeye salmon nursery lakes is that increased $\delta^{15}N$ values are due to (1) the trophic level of the biotic sample and (2) the N in the salmon carcasses. We assume that no other processes are significantly affecting $\delta^{15}N$. The exclusiveness of these two factors is also implied by the components of mixing model equations (2) and (3).

Ecosystems where N derived from $N_2$ fixation is important have low $\delta^{15}N$ reflecting the atmospheric N source (Minagawa and Wada 1984) because $N_2$ from the atmosphere has a $\delta^{15}N = 0$,

by definition. Similarly, we found low $\delta^{15}N$ in control primary producers because (1) there is no significant MDN input, (2) there is little isotopic fractionation during $N_2$ fixation, the a priori input of new N, and (3) these systems are oligotrophic and consequently show very little isotopic fractionation from the N source. Thus, $TEM_1 = 0$ (discussed extensively in Kline et al. 1990) where $TEM_1$ is the $\delta^{15}N$ value of trophic level one organisms based solely on atmospheric $N_2$ in the isotope mixing model (see equation (2)). We can probably eliminate biogeochemical processes, other than trophic level or the MDN source, that could potentially enrich $^{15}N$. For example, there is empirical evidence that very large $^{15}N$ enrichment can occur through denitrification when the N source $\delta^{15}N = 0$ (Böttcher et al. 1990) and is suggested as a cause for elevated $\delta^{15}N$ in biota in Pyramid Lake, Nevada (Estep and Vigg 1985). Elevated $^{15}N$ occurs even though Pyramid Lake receives large quantities of atmospherically-derived N through pollution and is also known to have a large rate of $N_2$ fixation (Horne and Galat 1985). There are two factors that probably preclude denitrification from being a major factor affecting $\delta^{15}N$ in anadromous Pacific salmon habitats. (1) Denitrification occurs when dissolved $O_2$ is either low or absent. Because sockeye salmon are very sensitive to low $O_2$, they are not likely to inhabit lakes where denitrification is significant. (2) Anadromous sockeye salmon lakes must have an outlet to the sea. These lakes thus flush out, unlike terminal lakes that loose water by other means such as evaporation. Flushing of the system also reduces the opportunity for N recycling and concomitant shifts in isotopic composition of the dissolved N pool. The small range in $\delta^{15}N$ of $\pm 2$ per mil seen in our control system periphyton in this as well as our Sashin Creek study (Kline et al. 1990) could be due to isotopic shifts in dissolved N due to small occurrences of $^{15}N$-enriching biogeochemical processes such as denitrification, differences in isotopic discrimination between algal species, effect of growth rate on isotopic discrimination, or effects of using different inorganic N substrates by algae (Wada and Hattori 1991). Control periphyton $\delta^{15}N$ variation was thus assumed to represent variability due to isotopic fractionations arising from N cycling of originally atmospherically derived N as in Sashin Creek (Kline et al. 1990). The standard deviation of $\delta^{15}N$ for the collective control lake periphyton values was 1.2 per mil. However, this variance is small compared with the difference in $\delta^{15}N$ between atmospheric $N_2$ and salmon N. With a large enough sample, the central tendency in the data will suggest the $\delta^{15}N$ of the N source. Such is the case in our data sets collected in this study and in our previous study (Kline et al. 1990).

## Variation in Adult Sockeye Salmon $\delta^{15}N$ and $\delta^{13}C$

Sockeye salmon return to spawn after 2 or 3 yrs in the ocean (Foerster 1968). Although no difference was found in the chemical composition relative to ocean-age (Mathisen et al. 1988), the 3-ocean-age salmon are larger in size, potentially having shifted feeding to a higher trophic level, thus affecting their isotopic signature because of trophic enrichment of $^{15}N$ (Minagawa and Wada 1984). Mathisen et al. (1988) found that about 30% of the N inventory in adult sockeye salmon at entry into Iliamna Lake is released into freshwater as excretion and gametes prior to decomposition. We tested for variation in $\delta^{15}N$ of adult salmon during their final phase of life to determine whether the marine $\delta^{15}N$ signature varied with time as a result of this N loss or because of ocean age. Three approaches were taken in examining the variability of $\delta^{15}N$ in returning adult salmon: (1) 20 homogenized whole carcasses previously analyzed for chemical composition (Mathisen et al. 1988) were analyzed for isotopic shifts of the whole fish during maturation by spawning site, sex, and ocean age, (2) small tissue samples from individual carcasses were compared for $\delta^{15}N$ and $\delta^{13}C$ to identify sources of isotopic variation, and (3) evaluation of variation of adult salmon $\delta^{15}N$ and $\delta^{13}C$ was made by comparison of standardized subsamples. The isotopic analyses of salmon carcasses suggested only small shifts in $\delta^{15}N$ during the maturation process and very little variability within a population of sockeye salmon at point-of-entry into the lacustrine environment. Our results suggest that a maximum shift of 2.3 per mil in $\delta^{15}N$ of the marine N input would result from the differential release of $^{15}N$ and $^{14}N$ during the time course of maturation, probably from remobilization of amino acids (Mommsen et al. 1980). Thus, the early marine N pool released through excrement and gametes could be as isotopically light as +10.0 and the N pool released by decomposition as isotopically heavy as +12.3. Mature eggs ($\delta^{15}N = +12.3$) have more positive $\delta^{15}N$ than developing eggs ($\delta^{15}N = +11.6$), corroborating the relationship between the low $\delta^{15}N$ of initial N lost through excretion and gametogenesis. However, the $\delta^{15}N$ value of $+11.5 \pm 0.5$ (SD) represented the natural variability of the returning adults and thus the total imported marine N pool. This value was more applicable to analysis of the total effect of salmon N on a time-integrated basis greater than the July–August maturation period, where the effects of differential release of $^{15}N$ and $^{14}N$ would be seen in Iliamna Lake. This value is also consistent with high-seas-caught juvenile and mature sockeye salmon from the northern Gulf of Alaska (Welch and Parsons 1993). The biochemical process during remobilization of the C pool during maturation appears to generate a significant shift in $\delta^{13}C$. A consistent fractionation between lipids and other constituents effected a difference in 2 per mil between salmon egg and carcass $\delta^{13}C$. This distinctive difference in egg and carcass $\delta^{13}C$ has potential for distinguishing their consumption by scavengers in future studies.

## Relating $\delta^{15}N$ to MDN

With the $\delta^{15}N$ of N from the atmosphere = 0 (by definition), the $\delta^{15}N$ of N in primary producers (periphyton) arising from it also $= 0 \pm 1.2$, and the $\delta^{15}N$ of marine N $= +11.5 \pm 0.5$, a consistently large $\delta^{15}N$ range exists permitting $\delta^{15}N$ to be used to quantify MDN in freshwater habitats of anadromous Pacific salmon. The $\delta^{15}N$ data from this investigation and our previous study (Kline et al. 1990) suggest that MDN can be quantitatively traced into both lentic and lotic freshwater habitats of anadromous Pacific salmon. The relative variability of $\delta^{15}N$ in biota in control systems was small compared with the large difference between control and spawning habitat biota, suggesting that the isotopic signatures reflect MDN input. The greater variability of $\delta^{15}N$ of periphyton within spawning habitats seen in Iliamna Lake and Sashin Creek (Kline et al. 1990) is ascribed to the spatial and temporal heterogeneity of MDN input because of the close relationship of $\delta^{15}N$ with respect to local carcass abundance. The fractionation of the salmon N pool from +11.5 to +7 by primary producers appears to be consistent in lentic and lotic systems. As previously discussed (Kline et al. 1990), the greatest isotopic fractionation between inorganic N and primary producers occurs when the inorganic N concentration is high. The lowest $\delta^{15}N$ expected (i.e., maximum fractionation) from an alga using MDN exclusively is +5 (Kline et al. 1990). The higher $\delta^{15}N$ value of +7 used in our mixing models (Kline et al. 1990, Fig. 1)

and the equivalent, equation (3) here, is consistent with the moderately oligotrophic conditions of Iliamna Lake, i.e., measured $NO_3^- \sim 5$ μM (Mathisen et al. 1988). Because the greatest variation in $\delta^{15}N$ of periphyton occurred at carcass drift sites and high spawning density sites, the variation is ascribed to MDN input reflecting the inhomogeneous nature of carcass distribution and thus nutrient input. The mean $\delta^{15}N$ of ~+7 is thus the best estimate we can make for the primary producer marine end member ($MEM_{TL=1}$ in equations (2) and (3)) at this time. The mean $\delta^{15}N$ of oceanic N of +7 relative to atmospheric $N_2$ (Wada and Hattori 1991) parallels our observations of an isotopic enrichment of +7 for MDN versus terrestrial N in the biota in our studies. The analysis of consumer $\delta^{15}N$ data affirms the use of +7 as the primary producer marine end member. Because of the slower turnover time of chemical elements in fishes compared with primary producers, they, like other macrofauna, have isotope signatures that represent a time-integrated value of their diet (Wada et al. 1987). Although it is unlikely to find a freshwater-resident fish 100% dependent on MDN, eventually the accumulation of isotope data in connection with changes in MDN input via differences in escapements will provide a test of the mixing model through iterations with refinements in the end members. This will be shown best in the juvenile sockeye $\delta^{15}N$ data because of their specialized feeding behavior and consequent narrow $\delta^{15}N$ range at a given time.

The juvenile sockeye $\delta^{15}N$ data fluctuated within the range expected for a fixed TL = 3 and the resultant model constraints of +6.8 and +13.8 for 0 and 100% MDN, respectively. Preliminary sockeye fry data from a similar study conducted at Karluk Lake, Kodiak Island, suggest a higher dependence on MDN. Karluk sockeye fry range from +11.8 to +13.2 depending on escapement size, suggesting that anadromous salmon contribute 71–91% of the N pool, respectively (T.C. Kline et al., unpublished data). Karluk Lake is known for formerly having produced a large number of salmon for its size (Koenings and Burkett 1987b) and has been thought to be dependent on the nutrient loading derived from carcasses (Barnaby 1944). A further test to verify the $\delta^{15}N$ mixing model would be to measure the $\delta^{15}N$ of sockeye fry from a system with a small salmon run, which should have a low dependence of MDN, and therefore should have $\delta^{15}N$ values approaching +6.8. The high MDN estimated with $\delta^{15}N$ implies that marine-derived biogenic nutrients may be significant at lower escapements than estimated by mass balance of P, e.g., the 25 million spawners for 50% marine-derived P (Donaldson 1967).

## Primary Pathway Transferring MDN into Lacustrine Food Webs

Our data suggest that the role of direct consumption of MDN as allochthonous marine organic matter (eggs and fry) is not very significant, collectively, in Iliamna Lake fishes. Thus, remineralized MDN, by autochthonous production supporting food webs, is the process whereby returning anadromous salmon effect nutrient flow in this freshwater ecosystem, providing strong evidence supporting the hypothesis of a biogenic nutrient feedback loop from adult to juvenile sockeye salmon through their freshwater forage (Mathisen 1972). Because sockeye forage has been identified as a limiting factor (Koenings and Burkett 1987b), the nutrient feedback loop may be an important regulatory process for controlling sockeye freshwater production.

## Implications to Cyclic Dominance

Eggers and Rogers (1987) found that the Kvichak sockeye run has an inherent tendency to be cyclic in the absence of fishing. The fishing activity results in the amplification of the population cycling. They suggested that cycling results from interaction of offspring from peak and postpeak year classes. This can only occur during their lacustrine life history stage. Several mechanisms for this interaction can be postulated: competition, cannibalism, and predation. The first two mechanisms are a direct interaction, while the third mechanism is indirect and depends on the differential effects of predation with respect to age of fry. The stable isotope data suggest that predation on eggs and fry supplies only a small part of the diet of potential predators. Nevertheless, suggested year-to-year differences in consumption of fry by coastrange sculpin (Table 8) are in agreement with a differential predation hypothesis. Cannibalism of emerging fry is not supported by the isotope data of yearling sockeye. Yearling sockeye captured in the vicinity of where fry had recently emerged (littoral) had $\delta^{15}N$ and $\delta^{13}C$ of +11.8 and −27.7, respectively (Table 8), which are inconsistent with the expected values for a fry predator, +15.6 and −22.5, respectively. Evidence for competition for food (zooplankton) as a mechanism for intercohort interaction is the similarity in isotope signatures for concurrent broodlines of sockeye fry (Table 8), suggesting a common dietary source that is principally limnetic production. The rapid drop in MDN during 1986–87 following two successive strong runs of 1984–85 (Table 7) suggests that MDN is rapidly sequestered into the biomass of fry. The consumption of productivity by a strong cohort would be felt by the subsequent cohort if food resources were depleted. The "year-after effect" is in agreement with the conclusion of Eggers and Rogers (1987). Apparently the peak brood years are able to outcompete (in the Darwinian sense) the successive brood year, as is borne out by the relative poor survival of the postpeak cohort to smolt and recruitment to the fishery (Eggers and Rogers 1987). The postpeak year class in this study (1985) had an anomalously high survival rate of 2.4 returns per spawner ($R/S$), cf. <1 $R/S$ that returned from the postpeak years 1961 and 1980 (data from the Commercial Fisheries Division, Alaska Department of Fish and Game, King Salmon, Alaska). A more definitive postpeak year class (e.g., $R/S = 1.8$, Eggers and Rogers 1987) might show more conclusive evidence of the mechanism(s) discussed above using stable isotope ratio data other than those found here.

## Summary

The isotopic composition of juvenile Pacific salmon and other biota in freshwater habitats with anadromous salmon differs consistently from that in habitats without anadromous salmon (this study and Kline et al. 1990). The contrast in $\delta^{15}N$ is a "natural tracer" allowing us to use it as a proxy to quantify marine-derived biogenic nutrients transported to freshwater streams by the large terminal spawning migrations of Pacific salmon. Variation in $\delta^{15}N$ due to N cycling is significantly less than variation in $\delta^{15}N$ due to salmon N. The small-scale variations that are due to N cycling and, to a greater extent, the point-source variability in salmon carcass abundance are time integrated by consumers: fishes. Because of the specialized nature of sockeye salmon fry diet, their $\delta^{15}N$ was the best indicator of short-term (months) integrated variation in MDN in the lacustrine ecosystem, which was found to be dependent on size of escapement. MDN benefits lacustrine consumers primarily from limnetic food webs via remineralization.

## Acknowledgements

This work was supported by a grant from the National Science Foundation Division of Polar Programs (DPP 844112285), Alaska Sea Grant (NA86AA-D-SG041 and M/81-01), and by a student research fellowship awarded to T.C. Kline by the University of Alaska Water Research Center. We are grateful for the technical support provided by Brenda Holladay in the cryodistillation of samples in preparation for mass spectrometry and Norma Haubenstock for the mass spectrometry. Additional laboratory support came from Brenda Anderson, Richard Anderson, and Della Scalan at the University of Texas Marine Science Institute at Port Aransas. Steve Parker, Thomas Rogers, and several undergraduate students from the University of Washington Fisheries Research Institute, Iliamna, provided logistical support and assisted in the sampling. We thank Donald E. Rogers for use of the Fisheries Research Institute, Porcupine Island field camp. Warner R. Lew and Rita O'Clair of the Juneau campus of the University of Alaska participated in Iliamna system sampling. Many residents of the Iliamna region have provided assistance. Tim and Nancy LaPorte, owners of Iliamna Air Taxi, Rose and Nels Hedlund of Knutson Bay, Ray and Linda Williams of Pile Bay village, and Carl and Ruth Jensen of Pedro Bay village offered their homes and hospitality and provided other logistical support for our sampling there. Tricia Crandall, Brenda Schwantes, and Stacy Schwantes collected returning salmon at the Karluk wier. Lorrie D. Rea critically reviewed the early draft and provided useful improvements. Two anomymous reviewers provided beneficial suggestions for the final draft.

## References

BARNABY, J.T. 1944. Fluctuations in abundance of red salmon, *Oncorhynchus nerka* (Walbaum), of the Karluk River, Alaska. Fish. Bull. 50: 237–295.

BÖTTCHER, J., O. STREBEL, S. VOERKELIUS, AND H.-L. SCHMIDT. 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. J. Hydrol. 114: 413–424.

BURGNER, R.L., C.J. DICOSTANZO, R.J. ELLIS, G.Y. HARRY, JR., W.L. HARTMAN, O.E. KERNS, JR., O.A. MATHISEN, AND W.F. ROYCE. 1969. Biological studies and estimates of optimum escapements of sockeye salmon in the major river systems in southwestern Alaska. Fish. Bull. 67: 405–459

COLLIE, J.S., AND C.J. WALTERS. 1987. Alternative recruitment models of Adams River Sockeye salmon, *Oncorhynchus nerka*. Can. J. Fish. Aquat. Sci. 44: 1551–1561.

CRAIG, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12: 133–149.

DEMORY, R.L., R.F. ORRELL, AND D.R. HEINLE. 1964. Spawning ground catalog of the Kvichak River system, Bristol Bay, Alaska. U.S. Fish. Wildl. Serv. Spec. Sci. Rep. Fish. No. 488: 292 p.

DENIRO, M.J., AND S. EPSTEIN. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science (Wash., D.C.) 197: 261–263.

DONALDSON, J.R. 1967. The phosphorus budget of Iliamna Lake, Alaska, as related to the cyclic abundance of sockeye salmon. Ph.D. thesis, University of Washington, Seattle, Wash. 141 p.

EGGERS, D.M., AND D.E. ROGERS. 1987. The cycle of runs of sockeye salmon (*Oncorhynchus nerka*) to the Kvichak River, Bristol Bay: cyclic dominance or depensatory fishing? *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 343–366

ESTEP, M.L.F., AND S. VIGG. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake system, Nevada. Can. J. Fish. Aquat. Sci. 42: 1712–1719.

FOERSTER, R.E. 1968. The sockeye salmon, *Oncorhynchus nerka*. Bull. Fish. Res. Board Can. 162: 422 p.

FRY, B. 1969. Sources of carbon and sulfur nutrition for consumers in three meromictic lakes of New York state. Limnol. Oceanogr. 31: 79–88.

FRY, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. 33: 1182–1190.

FRY, B. AND E.B. SHERR. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. 27: 13–47.

GARD, R., B. DRUCHER, AND R. FAGEN. 1987. Differentiation of subpopulations of sockeye salmon (*Oncorhynchus nerka*), Karluk River system, Alaska. *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 408–418.

HERCZEG, A.L. 1987. A stable carbon isotope study of dissolved inorganic carbon cycling in a softwater lake. Biogeochemistry. 4: 231–263

HOAG, S.H. 1972. The relationship between the summer food of juvenile sockeye salmon, *Oncorhynchus nerka*, and standing stock of zooplankton in Iliamna Lake, Alaska. Fish. Bull. 70: 355–362.

HORNE, A.J., AND D.L. GALAT. 1985. Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. Limnol. Oceanogr. 30: 1229–1239.

HYATT, K.D., AND J.G. STOCKNER. 1985. Responses of sockeye salmon (*Oncorhynchus nerka*) to fertilization of British Columbia coastal lakes. Can. J. Fish. Aquat. Sci. 42: 320–331.

JUDAY, C., W.H. RICH, G.I. KEMMERER, AND A. MANN. 1932. Limnological studies of Karluk Lake, Alaska 1926–1930. Fish. Bull. 47: 407–436.

KLINE, T.C. JR. 1991. The significance of marine-derived biogenic nitrogen in anadromous Pacific salmon freshwater food webs. Ph.D. thesis, University of Alaska Fairbanks, Fairbanks, Alaska. 114 p.

KLINE, T.C. JR., J.J. GOERING, O.A. MATHISEN, P.H. POE, AND P.L. PARKER. 1990. Recycling of elements transported upstream by runs of Pacific salmon: I. $\delta^{15}N$ and $\delta^{13}C$ evidence in Sashin Creek, southeastern Alaska. Can. J. Fish. Aquat. Sci. 47: 136–144.

KOENINGS, J.P., AND R.D. BURKETT. 1987a. Population characteristics of sockeye salmon (*Oncorhynchus nerka*) smolts relative to temperature regimes, euphotic volume, fry density, and forage base within Alaskan lakes. *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 216–234.

KOENINGS, J.P., AND R.D. BURKETT. 1987b. An aquatic Rubic's cube: restoration of the Karluk Lake sockeye salmon (*Oncorhynchus nerka*). *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 419–434.

KROHKIN, E.M. 1967. Influence of the intensity of passage of the sockeye salmon *Oncorhynchus nerka* (Wald.) on the phosphate content of spawning lakes. Izdanija "Nauka," Leningrad 15: 26–31. (Transl. from Russian by Fish. Res. Board Can. Trans. Ser. 1273, 1968).

LAZERTE, B.D., AND J.E. SZALADOS. 1982. Stable carbon isotope ratio of submerged freshwater macrophytes. Limnol. Oceanogr. 27: 413–418.

LOW, L.L. 1972. Chlorophyll *a*, phytoplankton, and primary production in Iliamna Lake, Alaska. M.S. thesis, University of Washington, Seattle, Wash. 101 p.

MATHISEN, O.A. 1972. Biogenic enrichment of sockeye salmon lakes and stock productivity. Verh. Int. Ver. Limnol. 18: 1089–1095.

MATHISEN, O.A., P.L. PARKER, J.J. GOERING, T.C. KLINE, P.H. POE, AND R.S. SCALAN. 1988. Recycling of marine elements transported into freshwater by anadromous salmon. Verh. Int. Ver. Limnol. 23: 2249–2258.

MATHISEN, O.A., AND P.H. POE. 1981. Sockeye salmon cycles in the Kvichak River, Bristol Bay, Alaska. Verh. Int. Ver. Limnol. 21: 1207–1213.

MCPHAIL, J.D., AND C.C. LINDSEY. 1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173: 381 p.

MINAGAWA, M., AND E. WADA. 1984. Stepwise enrichment of $^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48: 1135–1140.

MIZUTANI, H., Y. KABAYA, AND E. WADA. 1991. Nitrogen and carbon isotope compositions relate linearly in cormorant tissues and its diet. Isotopenpraxis 27: 166–168.

MOMMSEN, T.P., C.J. FRENCH, AND P.W. HOCHACHKA. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can. J. Zool. 58: 1785–1799.

NORTHCOTE, T.G. 1988. Fish in the structure and function of freshwater ecosystems: a "top-down" view. Can. J. Fish. Aquat Sci. 45: 361–379.

PETERSON, B.J., AND R.W. HOWARTH. 1987. Sulfur, carbon and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32: 1195–1213.

POE, P.H. 1980. Effects of the 1976 volcanic ash fall on primary productivity in Iliamna Lake, Alaska, 1976–1978. M.S. thesis, University of Washington, Seattle, Wash. 210 p.

QUAY, P.D., S.R. EMERSON, B.M. QUAY, AND A.H. DEVOL. 1986. The carbon cycle for Lake Washington stable isotope study. Limnol. Oceanogr. 31: 596–611.

RAU, G. 1978. Carbon-13 depletion in a subalpine lake: carbon flow implications. Science (Wash., D.C.) 201: 901–902.

RAU, G. 1980. Carbon-13/carbon-12 variation in subalpine lake aquatic insects: food source implications. Can. J. Fish. Aquat. Sci. 37: 742–746.

RAVEN, J., J. BEARDALL, AND H. GRIFFITHS. 1982. Inorganic C-sources for *Lemanea*, *Cladophora* and *Rancunculus* in a fast-flowing stream: measurements of gas exchange and of carbon isotope ratio and their ecological implications. Oecologia 53: 68–78.

ROGER, P.B. 1971. The ecology of two species of cottids in Iliamna Lake, Alaska, and their relation to sockeye salmon. M.S. thesis, University of Washington, Seattle, Wash. 80 p.

WADA, E., AND A. HATTORI. 1991. Nitrogen in the sea: forms, abundances, and rate processes. CRC Press, Boca Raton, Fla. 208 p.

WADA, E., H. MIZUTANI, AND M. MINAGAWA. 1991. The use of stable isotopes for food web analysis. Crit. Rev. Food Sci. Nutr. 30: 361–371.

WADA, E., M. TERAZAKI, Y. KABAYA, AND T. NEMOTO. 1987. $^{15}N$ and $^{13}C$ abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. 34: 829–841.

WELCH, D.W., AND T.R. PARSONS. 1993. $\delta^{13}C$–$\delta^{15}N$ values as indicators of trophic position and competitive overlap for Pacific salmon (*Oncorhynchus* spp.). Fish. Oceanogr. 2: 11–23.

WILLIAMSON, F.S.L., AND L.J. PEYTON. 1962. Faunal relationships of birds in the Iliamna Lake area, Alaska. Biol. Univ. Pap. Alaska. No. 5: 72 p.

YOSIOKA, T., E. WADA, AND Y. SAIJO. 1989. Isotopic characterization of Lake Kizaki and Lake Suwa. Jpn. J. Limnol. 49: 119–128.

# Growth Zones within the Statolith Microstructure of the Deepwater Squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae): Evidence for a Habitat Shift?

George D. Jackson[1]

*Department of Marine Science, University of Otago, Portobello Marine Laboratory, P.O. Box 8, Portobello, New Zealand*

Jackson, G.D. 1993. Growth zones within the statolith microstructure of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae): evidence for a habitat shift? Can. J. Fish. Aquat. Sci. 50: 2366–2374.

Within the statoliths of the onychoteuthid squid *Moroteuthis ingens* captured off the southeast coast and the Chatham Rise (New Zealand) between 500 and 1239 m depth, there were conspicuous zones consisting of an inner opaque and outer translucent zone; these strikingly resembled zones observed in some teleost fish otoliths. There was no relationship between the number of increments within the opaque zone among individuals of different length and age, but the width of the opaque zone was negatively related to both mantle length and age. The smallest juveniles captured in bottom trawls had a narrow translucent zone relative to the opaque zone which suggested that the translucent zone is laid down after settlement and that settlement takes place probably at or before 160 mm mantle length in this species. This study suggests that the transition observed within the statolith microstructure of *M. ingens* represents a habitat change from an epipelagic habitat to a demersal habitat. Evidence for the hypothesis is drawn from analogous features observed within teleost otolith microstructure and from available information on *Moroteuthis* and *Onykia*.

Les statolithes du calmar *Moroteuthis ingens*, de la famille des Onychoteuthidés, capturé au large de la côte sud-est et de l'archipel Chatham (Nouvelle-Zélande), entre 500 et 1 239 m de profondeur, comportaient des zones distinctes composées d'une zone intérieure opaque et d'une zone extérieure translucide; celles-ci ressemblent remarquablement à des zones observées sur les otolithes de certains téléostéens. Il n'existait aucune relation entre le nombre d'anneaux de croissance dans la zone opaque chez les individus de longueur et d'âge différents, mais la largeur de la zone opaque était liée négativement à la longueur du manteau et à l'âge. Chez les juvéniles les plus petits capturés par des chaluts de fond, la zone translucide était étroite par rapport à la zone opaque; il semble donc que la zone translucide soit déposée après l'installation dans la zone démersale et que cette installation se produit lorsque la longueur du manteau atteint 160 mm ou avant. La présente étude semble indiquer que la transition relevée dans la microstructure du statolithe de *M. ingens* représente un passage de l'habitat épipélagique à un habitat démersal. Les preuves de cette hypothèse sont tirées de caractères analogues observés dans la microstructure des otolithes des téléostéens et des informations obtenues sur *Moroteuthis* et *Onykia*.

*Received August 31, 1992*
*Accepted May 11, 1993*
*(JB613)*

*Reçu le 31 août 1992*
*Accepté le 11 mai 1993*

Pelagic squids are an ecologically important group within the oceanic ecosystem. They have many features such as their complex sensory structures and their raptorial mode of feeding which equip them to feed efficiently and to compete successfully with their vertebrate counterparts, the teleost fishes. Oceanic squids show many similarities to epipelagic and mesopelagic teleosts: they are fast swimming, voracious predators, and some undertake extensive migrations (Dunning 1988). From an ecological and trophodynamic perspective, pelagic squids are more closely related to fish than to other groups of Mollusca or even to other marine invertebrates. Moreover, the daily increments within statoliths of both nearshore and oceanic squids are analogous to increments within the otoliths of juvenile and some adult fishes.

In recent years, statoliths have been used to obtain useful information on the periodicity of growth increments (e.g., Jackson 1990b; Rodhouse and Hatfield 1990a; Nakamura and Sakurai 1991; Bigelow 1992), growth and life history (e.g., Jackson 1990a; Rodhouse and Hatfield 1990b; Bigelow 1992; Jackson and Choat 1992), and reproductive tactics (Jackson 1993) for a number of nearshore and oceanic squid species. Nevertheless, little work has been carried out on the significance of growth zones within the statolith microstructure. However, Bigelow (1992) has recently obtained information on a change in the pattern of statolith growth in paralarval *Abralia trigonura*, based on an abrupt change in both increment width and a shift in the growth axis of the statolith. This change was related to a migration from the epipelagic to the mesopelagic habitat coupled with the onset of diel vertical migration. Thus, there appears to be potential for obtaining information recorded within the statolith microstructure that may give clues to an individual's life history.

Much has been revealed about the life history of teleost fishes based on otolith analysis (Campana and Neilson 1985). Useful ecological information has been obtained by identifying a settlement check in species that take up a demersal habitat after a pelagic larval period (Victor 1982, 1986; Brothers et al. 1983; Fowler 1989; Cowen 1991; May and Jenkins 1992). Otolith

[1]Current address: Department of Zoology, University of Western Australia, Nedlands, Perth, Western Australia, 6009, Australia.

microstructure also contains information regarding past environmental and feeding conditions in some species (Campana 1984; Neilson and Geen 1985). It is therefore conceivable that similar past life history and environmental influences may be recorded in the squid statolith, especially for species that may undergo a marked habitat change during ontogenesis. Interpretation of such patterns within the statolith microstructure would be valuable for oceanic and deepwater species for which little biological information is available.

The onychoteuthid squid *Moroteuthis ingens* is an important item in the diet of sperm whales and seals but little is known of its biology (Roper et al. 1984). Lipinkski and Linkowski (1986) published preliminary information on the occurrence, allometry, and reproductive status of *M. ingens* in New Zealand waters and have suggested that statoliths of *M. ingens* might not be suitable for ageing. However, Arkhipkin and Bizikov (1991) have shown that enumeration of statolith increments is possible and have published length-at-age data for individuals of *M. ingens* captured on the Argentine continental slope. Tsuchiya and Okutani (1991) have proposed that the nominal genera *Moroteuthis* and *Onykia* may represent a confusing complex of growth stages of closely related species.

The statolith analysis in this study is part of a larger project on seasonal and geographic variation in age and growth of *M. ingens* in New Zealand waters (G.D. Jackson, in preparation). I document the growth zones within the statolith of *M. ingens*, comment on their significance, and suggest factors influencing their formation.

## Materials and Methods

Terminology for the statolith microstructure is after Lipinski et al. (1991). An "increment" is considered to be composed of both a dark and a light ring, and "zone" is used to describe a general region within the statolith.

Statolith microstructure was analysed in 14 individuals of *M. ingens* from four localities off the southeast coast of New Zealand (between 46°36.5′S, 170°14.5′E and 46°43.7′S, 170°07.0′S) in March 1992 collected using a bottom trawl (30-m wingspan, 5-m headline height with small ground rollers) in 500–534 m water depth. Six individuals were also obtained from four localities on the northern slopes of the Chatham Rise (between 42°48.39′S, 179°22.6′W and 42°36.59′S, 176°33.39′E) in June 1992 using a bottom trawl with a 5.0- to 6.4-m headline height. Individuals captured on the Chatham Rise were from greater depths (842– 1239 m) compared with those captured off the southeast coast. All squid were frozen upon capture.

In the laboratory, squid were defrosted (usually under running seawater). Mantle length was measured to the nearest millimetre. To obtain statoliths, the funnel was cut away from the posterior head region and a transverse incision was made through the paired statocysts in the posterior region of the cephalic cartilage. The head was then bent back to expose the statocysts and the statoliths lying inside. The statoliths were removed with fine forceps and placed directly on microscope slides and rinsed with 95% ethanol. In most instances the right statolith was analysed; however, the left one was used if the right one was unavailable.

For microstructural analysis, the statolith was mounted in the thermoplastic cement Crystal Bond (see Jackson 1990a) and positioned with the anterior (concave) surface up. Grinding was carried out using wet 1200-grade carborundum paper until a flat surface was obtained out to the periphery of the dorsal dome. The ground surface was then polished by hand on a wet felt (Leco Lecloth) impregnated with 0.05-μm alumina powder. After grinding, growth increments could be observed on the distal regions of the dorsal dome. However, to reveal growth increments in the nuclear region, the statolith was turned over and ground and polished on the convex surface until the grinding plane extended approximately two thirds of the way to the margin of the dorsal dome. Microstructural analysis and increment counts were then carried out on the posterior (convex) surface, as the nuclear region was usually only visible when viewed from the posterior surface, even after grinding and polishing both surfaces of the statolith.

The statolith microstructure of *M. ingens* consists of two prominent zones (an opaque and a translucent zone; see Results). The number of increments in each zone was determined by taking the mean of three counts (400× magnification) which varied less than 10% of the mean. The total number of increments within the statolith was determined by summing the mean increment counts of both zones. In one specimen the outermost increments of the dorsal dome were not visible and these were extrapolated as in Jackson and Choat (1992). The extrapolated increments were less than 5.2% of the total increment number. Counting of increments commenced from a prominent check in the nuclear region. The radius of the first check (from the focus to the margin, in the direction of the dorsal dome) was consistent among individuals (G.D. Jackson, in preparation).

The maximum width of each of the zones within the statolith was also measured with an eyepiece micrometer (200× magnification). The width of the opaque zone was taken from the focus to the outer margin of this zone, while the width of the translucent zone was taken from the outer margin of the opaque zone to the margin of the dorsal dome.

## Results

The statoliths of *M. ingens* varied considerably in shape: some had distinct dorsal and lateral domes (Fig. 1a and 1b), while others did not have a distinct demarcation between domes (Fig. 1c, 1d, 2a, and 2b). The majority of statoliths examined had two distinct zones, an inner opaque zone and an outer translucent zone, which extended to the statolith margin (Fig. 1 and 2). In statoliths from three individuals, the boundary between the two zones could not be precisely delineated; therefore, they were not used in the analysis. In one individual, no obvious zonation could be discerned and it was also not used.

Discontinuities (checks) occurred in both zones, but they were usually more common in the opaque zone. Near the outer margin of the opaque zone in several individuals, there were very regularly spaced checks which stopped abruptly at the edge of the opaque zone (Fig. 2a and 2b). Often there was also a prominent check designating the boundary between the opaque and the translucent zone (Fig. 1a, 1b, 2a, and 2b).

Statoliths from the two smallest juveniles (mantle length = 161 and 168 mm) obtained from the Chatham Rise consisted of predominantly an opaque zone and only a narrow translucent zone, with only eight and six increments, respectively, in the translucent zone (Fig. 2c).

### Increment Number in Zones

The number of increments within the opaque zone ranged from 112 to 209 (mean = 164.8, SD = 22.87, $n = 20$). In contrast, the number of increments in the translucent zone was much more

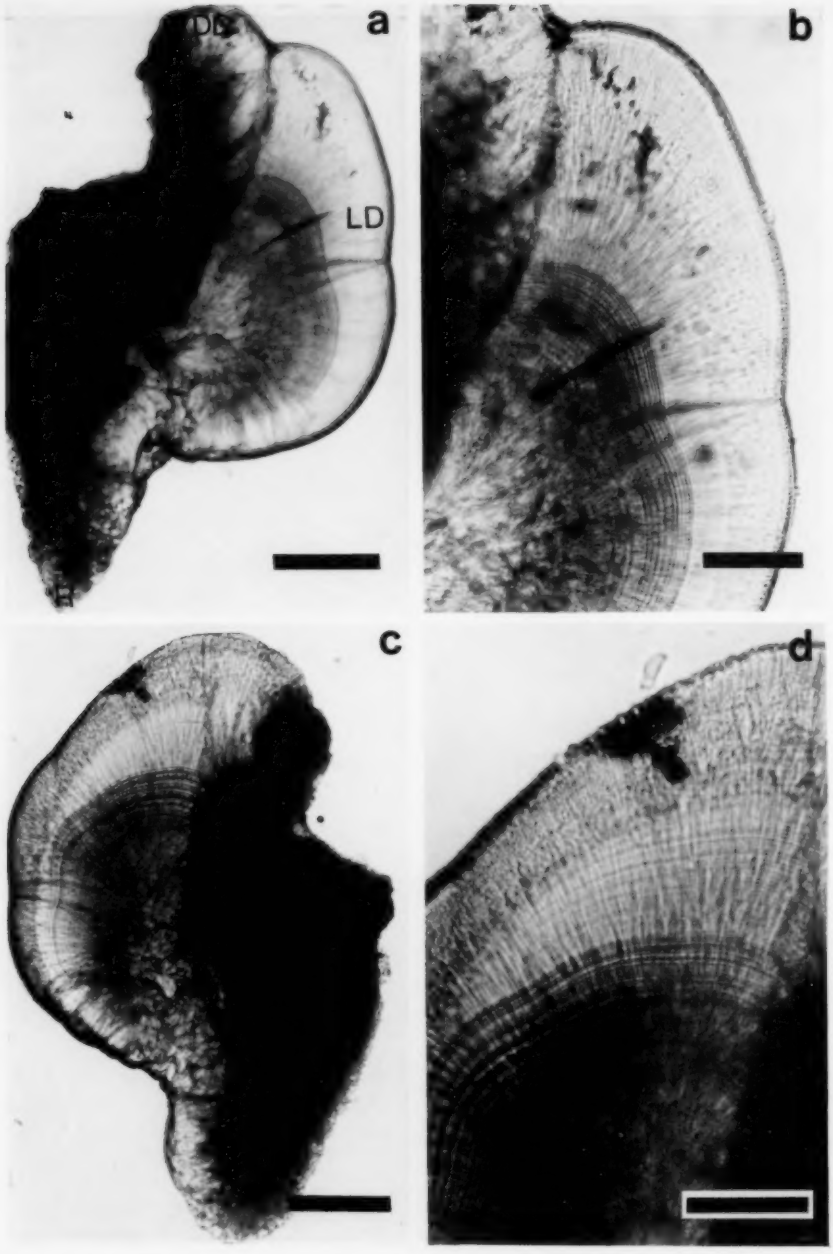



FIG. 1. Statolith microstructure of *M. ingens*. (a) Statolith of immature female, 360 mm ML, 256 total increments (DD = dorsal dome, LD = lateral dome, R = rostrum). Scale bar = 200 μm. (b) Close-up of statolith shown in Fig. 1a. Scale bar = 100 μm. (c) Statolith of mature male, 330 mm ML, 315 total increments. Scale bar = 200 μm. (d) close-up of statolith shown in Fig. 1c. Scale bar = 100 μm.

variable: from 6 to 215 (mean = 107.35, SD = 58.97, $n$ = 20). There was a significant positive relationship in (1) mantle length versus the number of increments in the translucent zone and (2) mantle length versus total increment number (Fig. 3a; Table 1). There was also a significant positive relationship in total increment number versus the number of increments in the translucent zone (Fig. 3b; Table 2). However, the relationships between mantle length versus the number of increments in the opaque zone and total increment number versus the number of increments in the opaque zone were not significant (Fig. 3; Tables 1 and 2), indicating that the number of increments within the opaque zone was not due to size or age.

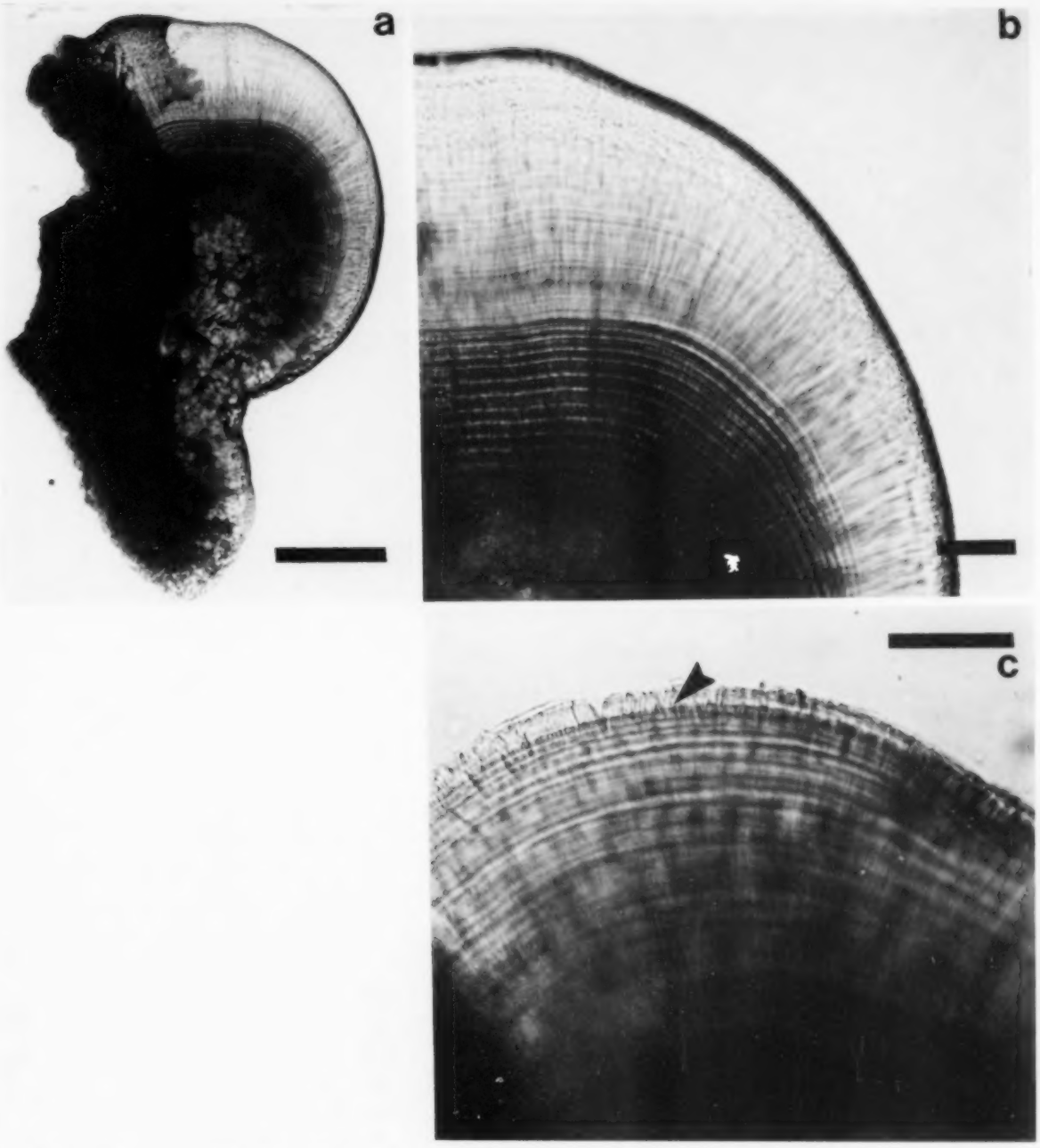



FIG. 2. Statolith microstructure of *M. ingens*. (a) Statolith of immature female, 345 mm ML, 302 total increments. Scale bar = 200 μm. (b) Close-up of statolith shown in Fig. 2a; note the series of regular checks leading up to the transition between the opaque and the translucent zone. Scale bar = 50 μm. (c) Dorsal dome region of statolith of juvenile male, 168 mm ML, 174 total increments; note the very narrow translucent zone at the margin (arrowhead indicates transition from opaque zone to translucent zone). Scale bar = 50 μm.

## Zone Widths

There was a significant positive relationship in (1) mantle length versus width of the translucent zone and (2) mantle length versus width from the focus to the margin (Fig. 4a; Table 3). The relationship was also positive between (1) total increment number versus width of the translucent zone and (2) total increment number versus width from the focus to the margin (Fig. 4b; Table 4). There were significant negative relationships between both mantle length versus width of the opaque zone and total increment number versus width of the opaque zone (Fig. 4;

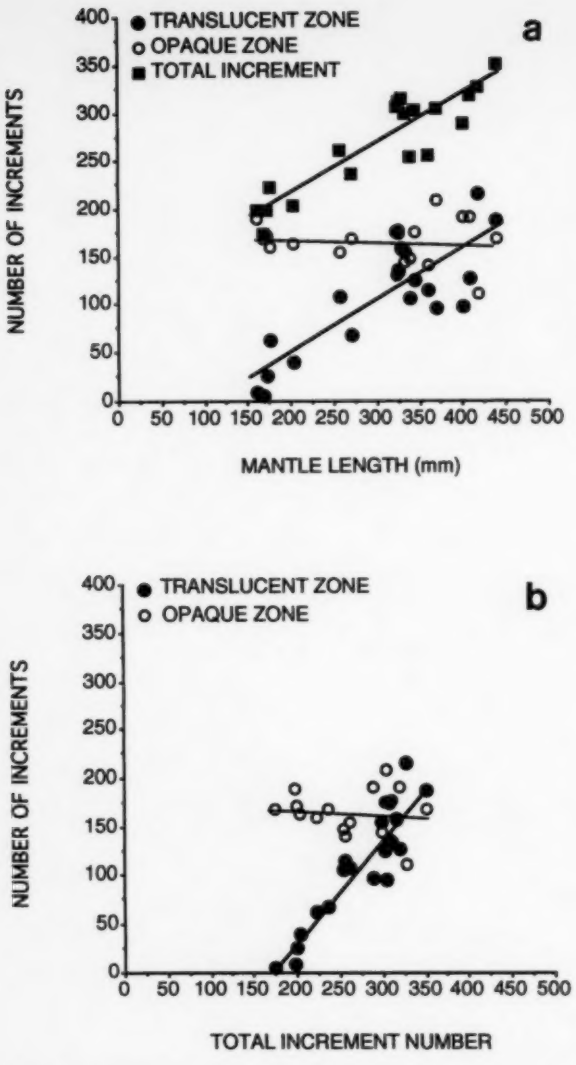



FIG. 3. Relationship between (a) mantle length and (1) the number of increments within the translucent zone, (2) the number of increments within the opaque zone, and (3) total increment number and (b) total increment number and (1) the number of increments within the translucent zone and (2) the number of increments within the opaque zone for all statoliths of *M. ingens* analysed.

Tables 3 and 4). Since the number of increments within the opaque zone was not related to size or age, the fact that the opaque zone was wider in the smaller (younger) individuals indicates that the growth increments within the opaque zone of the younger juveniles were wider than those of their larger (older) counterparts.

## Discussion

Optically distinct growth zones have been observed in statoliths of several oegopsid squid species. Kristensen (1980) described zones in statoliths of *Gonatus fabricii* based primarily

TABLE 1. Linear regression equations for the relationship between mantle length and the number of increments in the opaque zone, the number of increments in the translucent zone, and the total number of increments in both zones ($n = 20$).

| Zone | Equation | $r^2$ | $p$ |
|---|---|---|---|
| Opaque | $y = -0.02x + 172.06$ | 0.01 | 0.70 |
| Translucent | $y = 0.54x - 59.82$ | 0.69 | 0.0001 |
| Opaque + translucent | $y = 0.52x + 112.25$ | 0.81 | 0.0001 |

TABLE 2. Linear regression equations for the relationship between total increment number and the number of increments in the opaque zone and the number of increments in the translucent zone ($n = 20$).

| Zone | Equation | $r^2$ | $p$ |
|---|---|---|---|
| Opaque | $y = -0.41x + 176.04$ | 0.01 | 0.70 |
| Translucent | $y = 1.51x + 178.32$ | 0.85 | 0.001 |

on the visual intensity and spacing of growth increments. He identified a postnuclear zone which was described as light and uniform, followed by a dark zone and a third outer zone with prominent increments. He suggested that these zones might represent different maturity stages. Morris and Aldrich (1985) noted three zones in the statolith of *Illex illecebrosus*: R1, R2, and R3. R1 was translucent and thought to represent prehatching growth, R2 was opaque and thought to represent growth to maturity, and R3 was translucent and thought to represent mature growth. However, Balch et al. (1988) later showed that the first zone (R1) in *I. illecebrosus* was formed after hatching. Arkhipkin and Murzov (1990) commented on three zones within the statolith microstructure of *Abraliopsis atlantica* which they suggested were analogous to zones identified by Kristensen (1980) and Morris and Aldrich (1985). Based on the opaqueness and ring width, they associated these zones with growth during "larval and early juvenile," "adult immature," and "adult mature" stages. Distinct zonation in statolith microstructure has also been identified in *Sthenoteuthis pteropus* (Arkhipkin and Mikheev 1992). Arkhipkin and Bizikov (1991) also identified zones in statoliths of *M. ingens* from the Argentine continental slope (45–47°S). They observed that there was an initial opaque zone which could be divided into a "postnuclear" and "dark" zone based on increment widths. They also referred to a peripheral zone which they said was "transparent" unlike the previous two zones. They suggested that these zones related to different ontogenetic stages of growth ("larval," "juvenile," and "adult") in *M. ingens*.

No statolith growth zones have been observed in any tropical shallow-water, nearshore loliginid squids (Jackson 1990a, 1990b), or in the sepioid *Idiosepius pygmaeus* (Jackson 1989). Different growth zones have also not been reported for other temperate loliginids. For example, Natsukari and Komine (1992) found no trends in the microstructural appearance (spatial or density patterns) of increments for *Loligo vulgaris*, *Alloteuthis subulata*, *L. opalescens*, and *Photololigo edulis*.

None of the studies that have documented different zones within the statolith microstructure of oceanic squids have suggested an ambient mechanism for their formation. In the sections that follow, I propose some possible ambient environmental mechanisms for the development of the opaque zone and

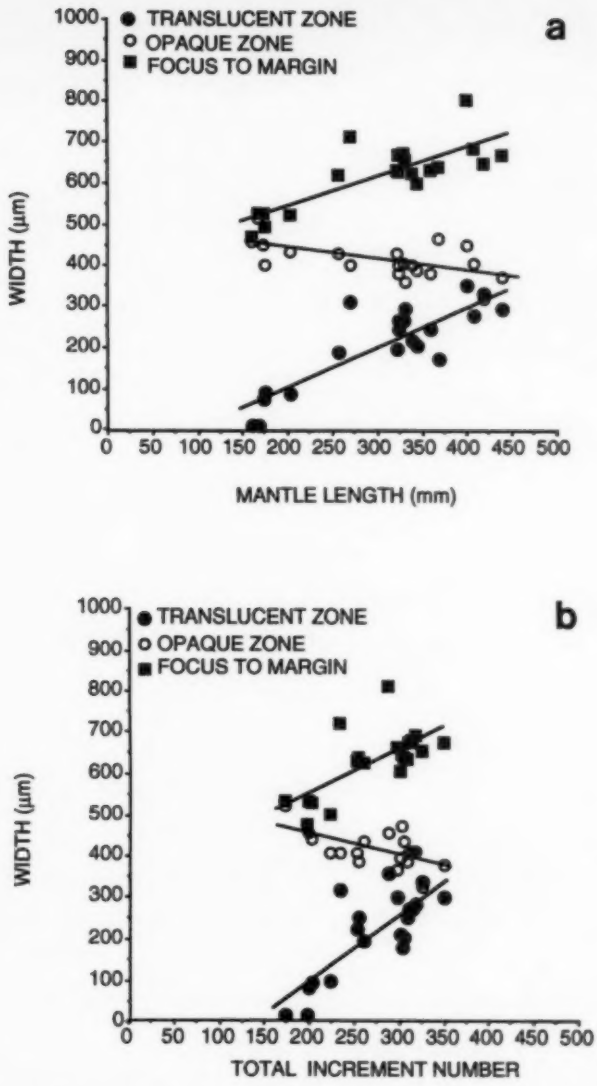



FIG. 4. Relationship between (a) mantle length and (1) the width of the translucent zone, (2) the width of the opaque zone, and (3) the width of the statolith radius (from the focus to the margin) and (b) total increment number and (1) the width of the translucent zone, (2) the width of the opaque zone, and (3) the width of the statolith radius (from the focus to the margin) for all statoliths of *M. ingens* analysed.

the translucent zone within the statolith microstructure of *M. ingens* in New Zealand waters. Evidence for the hypothesis is based on available information on the life history of *M. ingens*, ontogenetic differences in zone widths (and the number of increments within the respective zones), and parallel evidence of similar observed phenomena in fish otoliths.

## Zones in Fish Otoliths: Mechanisms for Their Production

Unfortunately, little is known of the biology and physiology of many deepwater and oceanic squids; however, possible ambient environmental influences governing statolith microstructure might be obtained by inference from research on teleost otoliths. There are some marked similarities between the statolith microstructure of *M. ingens* and the otolith microstructure of certain teleost species. The opaque and translucent zones within the statoliths of *M. ingens* resemble zones within the otoliths of mesopelagic lanternfishes (myctophids) (Gjosaeter 1987; Ozawa and Penaflor 1990; Gartner 1991a; Linkowski 1991); however, the nuclear area of the otolith of myctophids (representing growth during the larval period) is translucent. Gartner (1991a, 1991b) has suggested that these different zones are related to a habitat shift in the form of a downward migration (in which the opaque zone is laid down at depth, which is opposite to *M. ingens*).

Opaque and translucent zones have also been observed in otoliths of other nonoceanic fish species from widely different environments. A shift from an opaque to a more translucent microstructure has been recorded for chinook salmon (*Oncorhynchus tshawytscha*) (i.e., Neilson et al. 1985, fig. 2). This change was clearly related to a life history transition reflecting a shift from freshwater growth (opaque zone) to estuarine growth (more translucent zone with increments of higher contrast and greater width). Neilson et al. (1985) concluded that this change might be related to a greater amplitude in water temperature in the estuary relative to the river. Furthermore, Neilson and Geen (1985) were able to demonstrate that under experimental conditions, both the ambient temperature cycle and feeding conditions affected increment width and appearance in otoliths of *O. tshawytscha*. Starved individuals had a considerably more translucent otolith microstructure than did well-fed individuals. In commenting on differences (predominantly in increment width) observed within the otolith microstructure of chum salmon (*Oncorhynchus keta*), Volk et al. (1984) suggested that calcium deposition may be affected by a number of different factors such as quality and quantity of diet, temperature, and photoperiod.

Campana (1984) also noted different zones within the otolith microstructure of starry flounder (*Platichthys stellatus*). The transition from the inner to the outer zone was recognised by a change in both increment width and appearance, with the outer

TABLE 3. Linear regression equations for the relationship between mantle length and the width of the opaque zone, the width of the translucent zone, and the width of both zones from the focus to the statolith margin ($n = 20$).

| Zone | Equation | $r^2$ | $p$ |
|---|---|---|---|
| Opaque | $y = -0.27x + 500.37$ | 0.32 | 0.0087 |
| Translucent | $y = 1.0x - 95.64$ | 0.74 | 0.0001 |
| Focus to margin | $y = 0.72x + 404.73$ | 0.64 | 0.0001 |

TABLE 4. Linear regression equations for the relationship between total increment number and the width of the opaque zone, the width of the translucent zone, and the width of both zones from the focus to the statolith margin ($n = 20$).

| Zone | Equation | $r^2$ | $p$ |
|---|---|---|---|
| Opaque | $y = -0.52x + 556.64$ | 0.38 | 0.0038 |
| Translucent | $y = 1.60x - 225.03$ | 0.63 | 0.0001 |
| Focus to margin | $y = 1.08x + 331.61$ | 0.47 | 0.0008 |

zone having increased visual contrast. Campana (1984) noted that this transition was associated with metamorphosis. However, the physiological changes associated with metamorphosis alone were discounted as the factors influencing the appearance of the otolith, since otoliths of flounders which metamorphosed in the laboratory showed no change in visual contrast. However, it was concluded that concurrent shifts in behaviour and habitat (such as shifts in ambient temperature and food availability associated with a change from the pelagic to demersal habitat) could radically change the otolith microstructure. Campana (1984) concluded that "the transition zone in otoliths of wild flounders reflects an ecological (and not an internally derived physiological) shift in the animal's life history."

Shultz (1990) reported on microincrements laid down in otoliths of the viviparous dwarf perch (*Micrometrus minimus*) in two very different environments (interuterine versus shallow littoral). In this species, fish are "born" in an advanced stage of development and daily microincrements are laid down during uterine development as well as after "birth." The transition from the uterine environment to the nearshore environment is marked by a birthmark. Moreover, the otolith microstructure shows a marked change from opaque (during uterine development) to translucent (postbirth) (see Shultz 1990, fig. 7).

### Information on the Life History of *M. ingens*: An Interpretation of Statolith Zones

Based on the above evidence from teleost otolith research, it is likely that the transition from the opaque zone to the translucent zone within the statolith microstructure of *M. ingens* also represents a response to an ambient environmental influence rather than a phenomenon relating solely to an internal physiological process. I suggest that this transition may represent a habitat shift from an epipelagic existence to a demersal habitat. Available information on the life history of *Moroteuthis* is considered in light of the above information on zones in fish otoliths in the formation of this hypothesis.

Although little is known of the life history of many species of oceanic squids, we do know that they inhabit a wide variety of oceanic environments ranging from the near surface to great depths, while some species vertically migrate and others are demersal (Roper and Young 1975). Most oceanic oegopsid squids have planktonic paralarvae which generally inhabit near-surface waters (Roper and Young 1975; Young and Harmon 1988; Bigelow 1992). While some species may remain in epipelagic waters, many typically descend to the adult mesopelagic or bathypelagic habitat after spending an unknown time in near-surface waters (Vecchione 1987; Bigelow 1992).

*Moroteuthis ingens* appears to be a demersal species, as all specimens in this study were captured in bottom trawls (although capture of specimens during net deployment or retrieval cannot be discounted). Lipinski and Linkowski (1986) also recorded *M. ingens* from New Zealand waters in bottom trawls (450–1100 m). *Moroteuthis ingens* thus inhabits a similar environment to that of the demersal *M. robusta* in the Northern Hemisphere (Smith 1963; Roper and Young 1975). Very little is reported on the distribution of paralarval or juvenile *Moroteuthis* spp., although Dell (1952) recorded the capture of a juvenile *M. ingens* (20-mm mantle length) at the surface in northern New Zealand waters. Dubinina (1980) recorded two late paralarvae (at the surface and at 100 m) of *M. robsoni* in the southwest Atlantic. Rancurel (1976) also recorded the presence of juvenile *Moroteuthis* sp. in stomach contents of tuna feeding in the epipelagic waters in the Gulf of Papua. The reason for the lack of information on paralarval and juvenile specimens of *Moroteuthis* is possibly due to a misidentification of the paralarvae and juveniles. Tsuchiya and Okutani (1991) carried out a detailed study on both *Moroteuthis* and *Onykia* and linked these two genera, showing that *Onykia* may represent juvenile stages of *Moroteuthis*. Furthermore, one of the more common species, *Onykia carribaea*, is considered to be a species complex rather than a single species (Tsuchiya and Okutani 1991; Sweeney et al. 1992).

The fact that some previously identified *Onykia* may be juveniles of *Moroteuthis* sheds rather interesting light on the life history of *Moroteuthis*, as *O. carribaea* has been reported from numerous localities in the Atlantic, Indian, and Pacific oceans ranging from 45°N to 40°S (Clarke 1966; Sweeney et al. 1992). *Onykia* is also one of the most common paralarvae taken in surface samples of warm to tropical waters in some areas (Tsuchiya et al. 1991). Furthermore, *O. carribaea* is nearly always captured near the surface (Clarke 1966) both day and night (Roper and Young 1975), a behaviour that coincides with the habitat and behaviour of most paralarvae and juveniles rather than adult oceanic squids.

### Significance of Statolith Growth Zones and Future Research

If the opaque and translucent zones within the statolith of *M. ingens* represent pelagic and demersal growth, respectively, then the demarcation between these two zones may be analogous to settlement checks observed in fish otoliths (e.g., Victor 1982, 1986; Brothers et al. 1983; Fowler 1989; Cowen 1991; May and Jenkins 1992). The very prominent and often regular checks observed within the opaque zone of some specimens (e.g., Fig. 2a and 2b) may be analogous to postlarval zone "bands" in the opaque (postlarval) zone of some myctophids (Gartner 1991a, fig. 5b). Gartner (1991a) suggested that these bands which were interspersed within the increment structure may represent resting stages during the fish's ontogenetic downward migration.

If the statolith increments are formed daily as has been shown with a number of nearshore and several oceanic species (see Jackson 1990b; Nakamura and Sakurai 1991; Bigelow 1992), then the shift from a pelagic to a demersal existence takes place between 112 and 209 d and probably at or before about 160 mm mantle length. The difference in the appearance of the transition between the opaque and the translucent zone may reflect the duration or speed of the downward migration and subsequent assumption of a demersal existence (i.e., the demarcation between zones was very distinct in some specimens, while in others there was a less obvious transition). Specimens that have a marked change from the opaque zone to the translucent zone may represent individuals that "settled out" quickly. Evidence from the two smallest juveniles (161 and 168 mm mantle length, e.g., Fig. 2c) supports the hypothesis that the translucent zone is laid down after taking up a demersal existence. The width of the opaque zone was clearly not related to growth (i.e., the relationships between width of the opaque zone and mantle length and width of the opaque zone and age were not positive). However, the negative relationships between width of the opaque zone and mantle length, and width of the opaque zone and age may be related to seasonal or biogeographical allometric growth differences. These negative relationships were predominantly due to the smallest juveniles having wider opaque zones

(i.e., wider growth increments within the opaque zone). The small juveniles were captured at a different time of the year than the rest of the individuals analysed (June versus March), at a different locality (Chatham Rise versus southeast New Zealand), and at a greater depth. Seasonally induced allometric growth differences between mantle length and statolith length have been shown to exist in the nearshore tropical loliginind *Loligo chinensis* (Jackson 1991).

The fact that the transition from the opaque zone to the translucent zone within the statolith microstructure takes place in small juveniles (e.g., Fig. 2c) contradicts an earlier hypothesis that suggested that these zones are related to juvenile and adult growth phases (Arkhipkin and Bizikov 1991). Likewise, zones observed in other squid species (Kristensen 1980; Morris and Aldrich 1985; Arkhipkin and Murzov 1990) may be related to ambient environmental changes (possibly coupled with ontogenetic morphological changes) as in fish (e.g., Campana 1984) rather than solely to internal physiological processes.

I suggest that researchers compare the statolith microstructure in oceanic squid species that undergo a habitat shift from a pelagic to a demersal mode of existence with that in species that have a wholly pelagic life cycle. I propose that evidence of such a pronounced life history transition will be recorded in the statolith microstructure. It would also be of interest to compare the statolith microstructure of squids in which ontogenetic migration is abrupt with that in species in which a downward migration is more gradual (see Vecchione 1987). Oxygen isotope analysis of the respective zones would also give information on ambient temperatures in which each zone was deposited.

## Acknowledgements

This study would not have been possible without obtaining squids from observers on commercial fishing trips and research cruises conducted by the Ministry of Agriculture and Fisheries, Wellington. I am indebted to Rob Mattlin and Annette Atkins (MAF, Greta Point, Wellington) for organising the collection and transport of squids. I also thank Christine Jackson and Bob Rowley for manuscript criticism and John Gartner for useful comments during the preparation of the manuscript. This research was supported by the University of Otago, Department of Marine Science, and the Portobello Marine Laboratory. Tenure for this research was supported by a University of Otago Postdoctoral Fellowship.

## References

ARKHIPKIN, A.I., AND V.A. BIZIKOV. 1991. Comparative analysis of age and growth rates estimation using statoliths and gladius in squids, p. 19–33. *In* P. Jereb, S. Ragonese, and S. von Boletzky [ed.] Squid age determination using statoliths. Proceedings of the International Workshop held in the Istituto di Tecnologia della Pescae e del Pescato (ITPP–CNR), Mazara del Vallo, Italy, 9–14 October 1989. N.T.R.–I.T.P.P. Spec. Publ. No. 1: 128 p.

ARKHIPKIN, A.I., AND A. MIKHEEV. 1992. Age and growth of the squid *Sthenoteuthis pteropus* (Oegopsida: Ommastrephidae) from the Central-East Atlantic. J. Exp. Mar. Biol. Ecol. 163: 261–276.

ARKHIPKIN, A.I., AND S.A. MURZOV. 1990. Age and growth patterns of the micronektonic squid *Abraliopsis atlantica*. Biol. Morya (Vladivost.) 5: 19–25. (Transl. from Russian, Sov. J. Mar. Biol. 16: 254–260)

BALCH, N., A.S. SIROIS, AND G.V. HURLEY. 1988. Growth increments in statoliths from paralarvae of the ommastrephid squid *Illex* (Cephalopoda: Teuthoidea). Malacologia 29: 103–112.

BIGELOW, K.A. 1992. Age and growth in paralarvae of the mesopelagic squid *Abralia trigonura* based on daily growth increments in statoliths. Mar. Ecol. Prog. Ser. 82: 31–40.

BROTHERS, E.B., D. MCB. WILLIAMS, AND P.F. SALE. 1983. Length of larval life in twelve families of fishes at "One Tree Lagoon," Great Barrier Reef, Australia. Mar. Biol. 76: 319–324.

CAMPANA, S.E. 1984. Microstructural growth patterns in the otoliths of larval and juvenile starry flounder, *Platichthys stellatus*. Can. J. Zool. 62: 1507–1512.

CAMPANA, S.E., AND J.D. NEILSON. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

CLARKE, M.R. 1966. A review of the systematics and ecology of oceanic squids. Adv. Mar. Biol. 4: 91–300.

COWEN, R.K. 1991. Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus pulcher*. Mar. Ecol. Prog. Ser. 69: 9–15.

DELL, R.K. 1952. The recent cephalopoda of New Zealand. Dom. Mus. Bull. 16: 157 p.

DUBININA, T.S. 1980. On a finding of larval *Moroteuthis robsoni* (Oegopsida, Onychoteuthidae) in south-west Atlantic. Zool. Zh. 59: 1094–1096.

DUNNING, M.C. 1988. Distribution and comparative life history studies of deepwater squid of the family Ommastrephidae in Australasian waters. Ph.D. thesis, University of Queensland, Queensland, Australia. 272 p.

FOWLER, A.J. 1989. Description, interpretation and use of the microstructure of otoliths from juvenile butterflyfishes (family Chaetodontidae). Mar. Biol. 102: 167–181.

GARTNER, J.V., JR. 1991a. Life histories of three species of lanternfishes (Pisces: Myctophidae) from the eastern Gulf of Mexico. I. Morphological and microstructural analysis of sagittal otoliths. Mar. Biol. 111: 11–20.

GARTNER, J.V. JR. 1991b. Life histories of three species of lanternfishes (Pisces: Myctophidae) from the eastern Gulf of Mexico. II. Age and growth patterns. Mar. Biol. 111: 21–27.

GJOSAETER, H. 1987. Primary growth increments in otoliths of six tropical myctophid species. Biol. Oceanogr. 4: 359–382.

JACKSON, G.D. 1989. The use of statolith microstructures to analyze life-history events in the small tropical cephalopod *Idiosepius pygmaeus*. Fish. Bull. 87: 265–272.

JACKSON, G.D. 1990a. Age and growth of the tropical nearshore loliginid squid *Sepioteuthis lessoniana* determined from statolith growth ring analysis. Fish. Bull. 88: 113–118.

JACKSON, G.D. 1990b. The use of tetracycline staining techniques to determine statolith growth ring periodicity in the tropical loliginid squids *Loliolus noctiluca* and *Loligo chinensis*. Veliger 33: 389–393.

JACKSON, G.D. 1991. Age, growth and population dynamics of tropical squid and sepioid populations in waters off Townsville, North Queensland, Australia. Ph.D. thesis, James Cook University of North Queensland, Queensland, Australia. 221 p.

JACKSON, G.D. 1993. Seasonal variation in reproductive investment in the tropical loliginid squid *Loligo chinensis* and the small tropical sepioid *Idiosepius pygmaeus*. Fish. Bull. 91: 260–270.

JACKSON, G.D., AND J.H. CHOAT. 1992. Growth in tropical cephalopods: an analysis based on statolith microstructure. Can. J. Fish. Aquat. Sci. 49: 218–228.

KRISTENSEN, T.K. 1980. Periodical growth rings in cephalopod statoliths. Dana 1: 39–51.

LINKOWSKI, T.B. 1991. Otolith microstructure and growth patterns during the early life history of lanternfishes (family Myctophidae). Can. J. Zool. 69: 1777–1792.

LIPINSKI, M., E. DAWE, AND Y. NATSUKARI. 1991. Introduction, p. 77–81. *In* P. Jereb, S. Ragonese, and S. von Boletzky [ed.] Squid age determination using statoliths. Proceedings of the International Workshop held in the Istituto di Tecnologia della Pesca e del Pescato (ITPP–CNR), Mazara del Vallo, Italy, 9–14 October 1989, N.T.R–I.T.P.P. Spec. Publ. No. 1: 128 p.

LIPINSKI, M.R., AND T.B. LINKOWSKI. 1986. Some aspects of the biology of squid *Moroteuthis ingens* (Onychoteuthidae) from New Zealand waters. Rep. Ses. Fish. Inst. 21: 97–105.

MAY, H.M.A., AND G.P. JENKINS. 1992. Patterns of settlement and growth of juvenile flounder *Rhombosolea tapirina* determined from otolith microstructure. Mar. Ecol. Prog. Ser. 79: 203–214.

MORRIS, C.C., AND F.A. ALDRICH. 1985. Statolith length and increment number for age determination of *Illex illecebrosus* (Lesueur, 1821) (Cephalopoda, Ommastrephidae). NAFO Sci. Counc. Stud. 9: 101–106.

NAKAMURA, Y., AND Y. SAKURAI. 1991. Validation of daily growth increments in statoliths of Japanese common squid *Todarodes pacificus*. Nippon Suisan Gakkaishi 57: 2007–2011.

NATSUKARI, Y., AND N. KOMINE. 1992. Age and growth estimation of the European squid, *Loligo vulgaris*, based on statolith microstructure. J. Mar. Biol. Assoc. U.K. 72: 271–280.

NEILSON, J.D., AND G.H. GEEN. 1985. Effects of feeding regimes and diel temperature cycles on otolith increment formation in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish. Bull. 83: 91–101.

NEILSON, J.D., G.H. GEEN, AND D. BOTTOM. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith

microstructure. Can. J. Fish. Aquat. Sci. 42: 899–908.

OZAWA, T., AND G.C. PENAFLOR. 1990. Otolith microstructure and early ontogeny of a myctophid species *Benthosema pterotum*. Nippon Suisan Gakkaishi 56: 1987–1995.

RANCUREL, P. 1976. Note sur les céphalopodes des contenus stomacaux de *Thunnus albacares* (Bonnaterre) dans le sud-ouest Pacifique. Cah. O.R.S.T.O.M. Ser. Oceanogr. 14(1): 71–80.

RODHOUSE, P.G., AND E.M.C. HATFIELD. 1990a. Age determination in squid using statolith growth increments. Fish Res. 8: 323–334.

RODHOUSE, P.G., AND E.M.C. HATFIELD. 1990b. Dynamics of growth and maturation in the cephalopod *Illex argentinus* de Castellanos, 1960 (Teuthoidea: Ommastrephidae). Philos. Trans. R. Soc. Lond. B 329: 229–241.

ROPER, C.F.E., AND R.E. YOUNG. 1975. Vertical distribution of pelagic cephalopods. Smithsonian Cont. Zool. 209: 51 p.

ROPER, C.F.E., M.J. SWEENEY, AND C.E. NAUEN. 1984. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. No. 125(3): 277 p.

SHULTZ, E.T. 1990. Daily otolith increments and the early life history of a viviparous fish, *Micrometrus minimus* (Embiotocidae). Copeia 1990(1): 59–67.

SMITH, A.G. 1963. More giant squids from California. Calif. Fish Game 49: 209–211.

SWEENEY, M.J., C.F.E. ROPER, K.M. MANGOLD, M.R. CLARKE, AND S.V. BOLETZKY. 1992. "Larval" and juvenile cephalopods: a manual for their identification. Smithson. Contrib. Zool. 513: 282 p.

TSUCHIYA, K., T. NAGASAWA, AND S. KASAHARA. 1991. Cephalopod paralarvae (excluding Ommastrephidae) collected from western Japan Sea and Northern sector of the east China Sea during 1987–1988: preliminary classification and distribution. Bull. Jpn Sea Natl. Fish. Res. Inst. 41: 43–71.

TSUCHIYA, K., AND T. OKUTANI. 1991. Growth stages of *Moroteuthis robusta* (Verrill, 1881) with the re-evaluation of the genus. Bull. Mar. Sci. 49: 137–147.

VECCHIONE, M. 1987. Juvenile ecology, p. 61–80. *In* P.R. Boyle [ed.] Cephalopod life cycles. Vol. II. Academic Press, London.

VICTOR, B.C. 1982. Daily otolith increments and recruitment in two coral-reef wrasses, *Thalassoma bifasciatum* and *Halichoeres bivattus*. Mar. Biol. 71: 203–208.

VICTOR, B.C. 1986. Delayed metamorphosis with reduced larval growth in a coral reef fish (*Thalassoma bifasciatum*). Can. J. Fish. Aquat. Sci. 43: 1208–1213.

VOLK, E.C., R.C. WISSMAR, C.A. SIMENSTAD, AND D.M. EGGERS. 1984. Relationship between otolith microstructure and the growth of juvenile chum salmon (*Oncorhynchus keta*) under different prey rations. Can. J. Fish. Aquat. Sci. 41: 126–133.

YOUNG, R.E., AND R.F. HARMON. 1988. "Larva", "paralarva" and "subadult" in cephalopod terminology. Malacologia 29: 201–207.

# Mate Preference in Anadromous and Landlocked Dolly Varden (*Salvelinus malma*) Females in Two Alaskan Streams

Koji Maekawa

*National Research Institute of Fisheries Science, Fish Ecology Section, Komaki 1088, Ueda, 386, Japan*

Teruaki Hino

*Applied Zoology Laboratory, Faculty of Agriculture, Hokkaido University, N9 W9, Sapporo, 060 Japan*

Shigeru Nakano

*Nakagawa Experimental Forest, Faculty of Agriculture, Hokkaido University, Otoineppu, Hokkaido, 098-25, Japan*

and William W. Smoker

*Division of Fisheries, School of Fisheries and Ocean Sciences, University of Alaska-Fairbanks, Juneau, AK 99801, USA*

Maekawa, K., T. Hino, S. Nakano, and W.W. Smoker. 1993. Mate preference in anadromous and landlocked Dolly Varden (*Salvelinus malma*) females in two Alaskan streams. Can. J. Fish. Aquat. Sci. 50: 2375–2379.

Observations made on spawning anadromous Dolly Varden (*Salvelinus malma*) demonstrated that anadromous females were aggressive toward stream-resident mature males but were never aggressive toward subordinate anadromous males. Landlocked females only attacked subordinate males smaller than the α male. The number of times that females spawned in a nest depended on the mating attempts of stream-resident and subordinate males. Such attempts discouraged females from ovipositing. Through this behaviour, female anadromous Dolly Varden possibly increase anadromy and fitness in offspring by choosing anadromous males; female landlocked Dolly Varden possibly increase the size and fitness of offspring by choosing relatively large males.

Des observations portant sur des géniteurs anadromes de Dolly Varden (*Salvelinus malma*) ont montré que des femelles anadromes étaient agressives à l'égard de mâles matures résidants du cours d'eau, mais qu'elles ne présentaient jamais d'agressivité à l'égard de mâles anadromes subalternes. Des femelles dulcicoles ne s'attaquaient qu'à des mâles subalternes plus petits que le mâle α. Le nombre de fois que des femelles frayent dans un nid dépendait des tentatives d'accouplement des mâles résidants et subalternes du cours d'eau. Ces tentatives n'incitaient pas les femelles à pondre. En adoptant ce comportement, les Dolly Varden femelles anadromes augmentent probablement le caractère anadrome et la valeur adaptative chez les descendants en choisissant des mâles anadromes; des Dolly Varden femelles dulcicoles augmentent probablement la taille et la valeur adaptative des descendants en choisissant des mâles d'assez grande taille.

*Received January 19, 1993*
*Accepted May 31, 1993*
*(JB759)*

*Reçu le 19 janvier 1993*
*Accepté le 31 mai 1993*

When life-history strategies vary among males, females should choose among potential males on the basis of the resources they control or on the genetic qualities they can provide for their young. For example, females might discriminate on the basis of age, defensive ability, or size, if these are indicators of resources or genetic quality (e.g., van den Berghe et al. 1989).

Little is known about whether female salmonids prefer males that use one of several alternative reproductive tactics or strategies (but see Sigurjonsdottir and Gunnarson 1989; Jarvi 1990). Alternative male reproductive tactics have been reported in a number of taxa, including salmonid fishes (see Alcock 1979; Clutton-Brock et al. 1982; Gross 1984). In forming mating pairs, relatively large salmonid males are superior in male–male competition and consequently, smaller or weaker males often become satellites or sneakers (Jones 1950; Hanson and Smith 1967; Schroder 1981; Maekawa 1983; Gross 1985; Keenleyside and Dupuis 1988; Sigurjonsdottir and Gunnarsson 1989; Hino et al. 1990). There is also evidence of indirect female choice, in that females spawn more readily with larger males (Schroder 1981; Foote and Larkin 1988; Foote 1989).

Size-polymorphic males occur in spawning populations of anadromous Dolly Varden (*Salvelinus malma*) (e.g. Maekawa and Onozato 1986; Maekawa and Hino 1987). Most or all of the females and a portion of the males are either anadromous or lake-run types that migrate downstream either to the sea or to a lake, returning as large individuals to the natal stream to spawn. The remainder are resident in the stream and much smaller in size than anadromous or lake-run fish.

The relative fitness of small and large males has not been evaluated. Resident small males engage in sneaking behaviour, in which they rush into the territory of anadromous or lake-run pairs and release semen simultaneously with large anadromous or lake-run males; it is unknown whether male types have equal fitness (but see Maekawa and Hino 1986). In contrast, the males of fluvial Dolly Varden (the landlocked form) are monomorphic and the seasonal mating success of individual dominant males is much higher than that of subordinate males (e.g., Hino et al.

TABLE 1. Mean length and mean age ± SD of Dolly Varden observed in mating groups in Steep Creek and Tiekel River, Alaska. Number ($n$) in parentheses.

| | Steep Creek | | Tiekel River, landlocked |
|---|---|---|---|
| | Anadromous | Stream resident | |
| Females | | | |
| Length (mm) | 420.8 ± 54.2 (27) | | 186.7 ± 27.0 (29) |
| Age (yr) | 6.4 ± 0.5 (14) | | 4.6 ± 0.7 (10) |
| Males | | | |
| Length (mm) | 415.3 ± 58.4 (14) | 205.0 ± 45.9 (6) | 171.4 ± 26.9 (76) |
| Age (yr) | 6.9 ± 0.8 (12) | 3.7 ± 0.9 (7) | 4.3 ± 1.3 (27) |

1990). Their mating tactics are conditionally determined depending on their size, with smaller males adopting the sneaking tactic (Hino et al. 1990).

When choices between males of different types are possible, females are expected to prefer anadromous males or larger males if they can produce larger and more dominant male offspring. Here, we describe the behaviour of anadromous and landlocked female Dolly Varden toward males and show that the former choose their own type of males and the latter choose the largest available males.

## Materials and Methods

Dolly Varden charr were observed spawning in Steep Creek, a small tributary of Mendenhall Lake, a glacial lake located near Juneau, Alaska (near 58°N, 135°W), on 1–10 October 1987 and 25 September to 7 October 1989 and in a small tributary of the Tiekel River in south-central Alaska (near 61°N, 145°W) on 15–28 September 1987. Dolly Varden in Steep Creek are typically anadromous, migrating upstream from the ocean to spawn; however, the population also includes a few stream-resident males (Armstrong and Morrow 1979; Maekawa and Hino 1986). The criteria of Maekawa (1983), including body colour, colour pattern, and body size, were used to distinguish anadromous males from resident ones. A small number of males that had intermediate characteristics were observed, and these individuals were categorized as resident males (2 of 38 individuals observed, 24 and 26 cm total length, respectively). Charr in the Tiekel River are landlocked, probably because gorges in the lower river prevent fish from returning from the ocean. In this river system, charr migrate from the main stream or ponds into small tributaries to spawn (Gregory 1988).

In Steep Creek, fish were captured by cast net and marked using ribbon tags. Few fish were captured and no lengths were directly taken. Instead, their body sizes (total length in centimetres) were determined by comparison with marks in the stream after a spawning activity was completed. Ages of individual fish in Steep Creek were determined from scales.

In Tiekel River, most of the fish were captured at the mouth with either a trap, a cast net, or a chase net. All the captured fish were individually marked with a combination of coloured ribbon tags after being measured (fork length in millimetres). Ages of individual fish in the Tiekel River were determined from otoliths (Hino et al. 1990).

Spawning behaviour was either recorded by a video tape recorder (VTR) or observed directly from the stream bank. Spawning groups were not disturbed by the observations.

In most cases, a spawning group consisted of one female and several males (Maekawa and Hino 1986; Hino et al. 1990). The dominance rank among males was determined on the basis of social interactions among the males (Hino et al. 1990). We defined the dominant male, i.e., the one that courted the female, as the "paired or α male," and subordinate males were categorized as "satellites."

The spawning behaviour of males from the Tiekel River and Montana Creek, a stream in the same watershed as Steep Creek, has been described previously by Hino et al. (1990) and Maekawa and Hino (1986), respectively.

Our present observations focus on (1) the size distribution of each spawning group, (2) the aggressive interactions of the female against males, and (3) the relationship between attempted sperm release by males (as recognized by a characteristic gaping of the mouth; Maekawa 1983; Maekawa and Hino 1986) and the behavioural response of females.

## Results

### Anadromous and Resident Dolly Varden in Steep Creek

Typically, an observed mating group of anadromous Dolly Varden consisted of a female and a paired male, zero to three anadromous satellite males ($\bar{x}$ = 1.53 ± 1.19 SD, $n$ = 15), and zero to three resident males ($\bar{x}$ = 0.86 ± 0.99 SD, $n$ = 15 groups) much smaller in body size and younger than the anadromous males (Table 1). The number of satellite males per pair increased with the body size of the paired male (Kendall's tau = 0.949, $p < 0.01$, $n$ = 13 spawning groups), but there was no relationship between the number of satellite males and the size of the female (Kendall's tau = 0.051, $p > 0.1$, $n$ = 13).

There was no significant difference between the average body size of females and males (Mann–Whitney's U-test, $p > 0.05$) (Table 1), yet in most pairs the male was larger than the female (Wilcoxon test, $p < 0.01$) (Fig. 1). No assortative mating for body size ($r$ = 0.202, $p > 0.05$, $n$ = 13) was found.

Females never attacked paired males and satellite males that were anadromous (656 min observed from recordings, seven spawnings, mean length of satellite males = 418.75 ± 49.10 SD, range = 300–470 mm, $n$ = 11). Females did attack resident males (mean number of times female attacked/min = 0.26 ± 0.53 SD, range = 0–1.818/min, $n$ = 8 spawning, mean length = 175.45 ± 47.61 SD, range = 120–260 mm, $n$ = 12). In stepwise multiple regression of attack frequency on satellite male size, there was no significant relationship between the frequency of attacks by a female and the satellite male's body size (Table 2).

Anadromous females also spawned one to three times in each nest (mean number of spawnings = 1.38 ± 0.76 SD, $n$ = 13) at

TABLE 2. Relationships in anadromous and landlocked spawning groups between frequency of attacks by spawning females on satellite males and length of satellite male, ratios of lengths of satellite and paired males, and ratios of lengths of satellite males and females. Correlation coefficients ($r$) and the results of stepwise multiple regression of female attack frequency on length of satellite males and ratios of lengths. Variables are tabled in order of contribution to $R^2$; $F$ value to enter variables in regressions was 2.0; cells are blank for variables not entering regressions. $^*p < 0.05$; $^{**}p < 0.01$.

| | | Stepwise regression (cumulative) | | |
|---|---|---|---|---|
| | Correlation coefficient ($r$) | Multiple correlation coefficient ($R$) | $R^2$ | Total $F$ |
| Anadromous females ($n$ = 22) | | | | |
| Length of satellite male | −0.280 | 0.280 | | 1.706 |
| Ratio of lengths, satellite male:paired male | −0.240 | | | |
| Ratio of lengths, satellite male:female | −0.232 | | | |
| Landlocked females ($n$ = 23) | | | | |
| Ratio of lengths, satellite male:paired male | −0.708** | 0.708 | 0.501 | 21.064** |
| Length of satellite male | −0.618* | | | |
| Ratio of lengths, satellite male:female | −0.557* | | | |

intervals of about 15 s. No significant relationship was found between the number of times a female spawned and the number of satellite males present (Kendall's tau = −0.051, $p > 0.05$, $n$ = 13). When resident males attempted to fertilize eggs, females stopped placing multiple batches of eggs into their nests (Table 3A). However, when anadromous satellite males participated in spawning, there was no apparent cessation of oviposition episodes.

### Landlocked Dolly Varden in Tiekel River

Satellite males do not appear to choose females by their body size. An average of 3.38 males (±1.25 SD, range = 1–6, n = 40 groups) aggregated around a spawning female (see Hino et al. 1990). The largest and the most dominant male (paired male) was beside the female and courted her. In a pattern similar to that observed around anadromous females at Steep Creek, there was a positive relationship between the number of satellite males per pair and the body size of the paired male (Kendall's tau = 0.291, $0.01 < p < 0.05$, $n$ = 27 spawnings), but no relationship existed between the number of satellite males and size of the paired female (Kendall's tau = 0.207, $p > 0.05$, $n$ = 27).

Paired males were generally larger than the females they courted (Wilcoxon test, $p < 0.01$) (Fig. 1), although females were larger than males on average (Mann–Whitney's U-test, $0.01 < p < 0.05$) (Table 1). No assortative mating for body size was observed ($r$ = 0.144, $p > 0.05$, $n$ = 19 from VTR). Nest construction and courting lasted for several hours and on a number of occasions, paired males were displaced by rivals. When larger males replaced smaller individuals, the female readily accepted the new male and sometimes attacked her former, smaller suitor. In three instances, however, larger males were displaced by smaller rivals. When this occurred, the female did not attack the larger, recently dislodged male. Usually, females attacked subordinate males smaller than themselves although in a few instances, satellite males larger than the female were attacked.

A stepwise multiple regression analysis was used to examine the importance of female body size, satellite body size, ratio of satellite male size to female size, and ratio of satellite male size to paired male size on the frequency of attacks a female directed toward a satellite male. This analysis suggested that the ratio of satellite male to paired male size was the most important factor regulating female attacks on subordinate males (Table 2). That is, the lower the dominance rank of a satellite male, the more frequently a female attacked him (Kendall's tau = 0.403, $p < 0.01$, $n$ = 32 from VTR and direct observations) (Fig. 2).

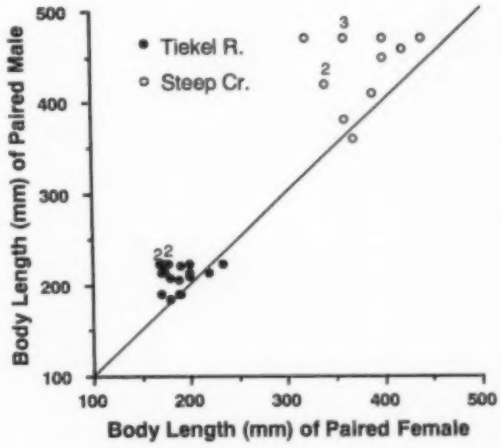



FIG. 1. Size of females and size of the dominant male (paired male) in spawning groups of anadromous and stream-resident Dolly Varden in Steep Creek and in spawning groups of landlocked charr in Tiekel River. Sizes of charr from Tiekel River and Steep Creek are shown as fork length and total length, respectively. The solid line indicates equality of sizes. Each symbol represents one spawning group unless otherwise labelled.

Females spawn from one to three times in each nest ($\bar{x}$ = 1.53 ± 0.69 SD, $n$ = 19) at intervals of about 10 s. No significant relationship was found between the number of times a female spawned and the number of satellite males at the nest (Kendall's tau = −0.137, $p > 0.05$, $n$ = 19). When at least one attendant satellite male attempted fertilization, however, the female discontinued oviposition (Table 3B). If no satellite male attempted fertilization, the female continued to a second or third oviposition.

TABLE 3. Female's response (continued or discontinued oviposition) to the behaviour of attendant satellite males (attempted fertilization or unsuccessful fertilization). Observations are of behaviour patterns between first and second ovipositions and between second and third (or potential third) ovipositions among (A) 23 ovipositions at 17 nests in the anadromous population of Steep Creek and (B) 27 ovipositions at 19 nests in the landlocked population of Tiekel River. The behaviour of satellite males is characterized as attempted fertilization when more than one of the attendant males showed this behaviour, regardless of the behaviour of the other males. Numbers in parentheses show attempted fertilization or unsuccessful fertilization of attendant satellite males.

| | Satellite males attempted fertilization | |
|---|---|---|
| | Yes | No |
| (A) Anadromous Steep Creek females' oviposition | | |
| Continued | 1 (1) | 8 (0) |
| Discontinued | 6 (4) | 2 (1) |
| Fisher's exact test, $p = 0.0202$ | | |
| (B) Landlocked Tiekel River females' oviposition | | |
| Continued | 3 | 9 |
| Discontinued | 12 | 3 |
| Fisher's exact test, $p = 0.0123$ | | |

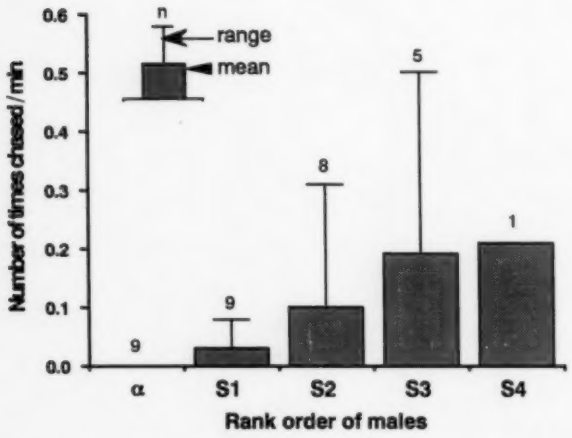



FIG. 2. Relationship between the number of times in which the satellite males are attacked by females and the rank order of satellite males in landlocked Dolly Varden. α: paired males; S1–S4: rank order (1–4) of subordinate satellite males.

## Discussion

Our results indicate that female Dolly Varden pair with males larger than themselves. This is mostly due to male–male competition (Maekawa and Hino 1986; Hino et al. 1990) which also plays an important role in pair formation in other charr and salmonid fishes (e.g., Jones 1959; Hanson and Smith 1967; Schroder 1981; Maekawa 1983; Gross 1985; Keenleyside and Dupuis 1988). A positive correlation between the size of a paired male and the number of satellite males surrounding a female is probably explained by male–male competition and/or the female's attractiveness coupled with escalating male competition. This shows that satellite males, by not selecting their mate (female), may increase their opportunities for spawning.

Female Dolly Varden directly attacked subordinate males. The target of female attacks, however, differed between anadromous and landlocked populations. Anadromous females attacked only resident males that aggregated around the nest; they never attacked anadromous males. This pattern has been observed in other anadromous populations of Dolly Varden (Maekawa and Hino 1986) and Miyabe charr (*Salvelinus malma miyabei*) that have a similar life history to anadromous charr (Maekawa 1977, 1984). Anadromous and lake-run females can discriminate between resident and anadromous males or lake-run males. Females probably discriminate by differences in coloration and size. Body colour of subordinate stream resident males quickly darkens after entering a spawning group; the colour of subordinate anadromous or lake-run males does not (e.g., Maekawa 1983). Landlocked females, however, selectively attacked subordinate males, apparently after comparing them with the size of their paired male.

We suggest that female anadromous Dolly Varden may increase anadromy among their offspring by choosing anadromous males. Further, female landlocked Dolly Varden, by choosing relatively large males in the spawning group, may increase the size of their offspring at maturity (e.g. Beacham 1988; Beacham and Murray 1988). These suggestions are supported by our observations that anadromous and landlocked females discriminate against fertilizations by stream-resident and subordinate males, respectively. However, we lack knowledge of the genetic variability of anadromy or of size, and our suggestion assumes that anadromy and size are heritable traits.

We note a relationship between female preference and the differences in alternative male reproductive strategies among charr. Female Miyabe charr apparently distinguish between fertilization and egg-eating attempts by resident males (Maekawa and Hino 1990). When all resident males exclusively adopt fertilization attempts, females tend to continue to deposit eggs in a nest, but if one male begins to eat eggs, a female will often discontinue spawning. Therefore, female Miyabe charr probably attack stream-resident males to avoid egg eating, not to discriminate against fertilization by stream-resident males. Because the resident males in Alaska have not been observed to

eat eggs, the cause of the female's attacks on stream-resident males may be different in anadromous Dolly Varden and Miyabe charr (Maekawa and Hino 1990).

The reproductive success and fitness of stream-resident males may be less than those of anadromous males, if most or all of stream-resident males never become anadromous, which is the case for Miyabe charr (Maekawa 1977; Maekawa and Onozato 1986). In Miyabe charr, both male types appear to have equal fitness (Maekawa and Hino 1986, 1987) and to exist in an evolutionarily stable state (mixed ESS's; Maynard Smith 1982) at the population level. In this species, females would not be expected to discriminate against either type of male because female mate preference is probably not free of cost (e.g., Foote 1989; but see Reynolds and Gross 1990). We suggested in an earlier paper that both male types of anadromous Dolly Varden in Alaska had equal fitness on the basis of some assumptions (Maekawa and Hino 1986). However, the mate preference of female anadromous Dolly Varden observed here, together with male–male competition, probably has a significant depressive effect on the mating success of stream-resident males. Consequently the stream-resident type may have relatively low fitness and has evolved as the best of a bad situation (BBS; Gross 1984). Female anadromous Dolly Varden would avoid such stream males and select anadromous males to produce as many offspring from anadromous males as possible.

Spawning tactics of landlocked males were conditionally determined, depending on body size and age at maturity. The mating success and probably also the fitness of subordinate males are much lower than those of largest and oldest dominant males (Hino et al. 1990; Kitano 1992; and personal communication), due to both female mate preference and male–male competition. If a female can influence her own offspring's fitness by choosing the largest male available, then she should be expected to avoid relatively small subordinate males. The effect of such female mate preference is supported by the recent demonstration that the progeny of large male pink salmon (*Oncorhynchus gorbuscha*), in which males adopt conditional tactics during spawning (Keenleyside and Dupuis 1988), grew faster and attained a greater size at maturity than that of small males (Beacham and Murray 1988).

In conclusion, both types of males probably adopt conditional tactics (BBS). Female Dolly Varden prefer males with relatively larger body sizes, but they appear to choose males with potentially high reproductive success. Female mate preference or choice may result in improving her own offspring's fitness and also have a significant effect on male mating success.

## Acknowledgements

We are very grateful to James B. Reynolds, S. Gregory, Mitsuru Saito, Satoshi Odachi, Mineo Saneyoshi, Hiroshi Kawamura, and Junko Terazaki for their assistance during the investigation. We also thank Hiroyuki Matsuda, Mart R. Gross, Yasushi Hashimoto, Osamu Katano, and M.J. Hutchisson for valuable comments on an earlier draft. The present research was supported by a grant-in-aid for the Over-seas Scientific Survey from the Ministry of Education, Science and Culture of Japan. This paper is contribution No. B71 from the National Research Institute of Fisheries Science.

## References

ALCOCK, J. 1979. The evolution of intraspecific diversity in male reproductive strategies in some bees and wasps, p. 381–403. *In* M.S. Blum and N.A. Blum [ed.] Sexual selection and reproductive competition in insects. Academic Press, New York, N.Y.

ARMSTRONG, R.H. AND T.E. MORROW. 1979. The Dolly Varden charr, *Salvelinus malma*, p. 19–98. *In* E.K. Balon [ed.] Charrs: salmonid fishes of the genus Salvelinus. Dr. W. Junk Publishers, The Hague, The Netherlands.

BEACHAM, T.D. 1988. A genetic analysis of early development in pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) at three different temperatures. Genome 30: 89–96.

BEACHAM, T.D., AND C.B. MURRAY. 1988. A genetic analysis of body size in pink salmon (*Oncorhynchus gorbuscha*). Genome 30: 31–35.

CLUTTON-BROCK, T.H., F.T. GUINESS, AND S.D. ALBION. 1982. Red deer: the behaviour and ecology of two sexes. University of Chicago Press, Chicago, Ill.

FOOTE, C.J. 1989. Female mate preference in Pacific salmon. Anim. Behav. 38: 721–722.

FOOTE, C.J., AND P.A. LARKIN. 1988. The role of male choice in assortative mating of anadromous and non-anadromous sockeye salmon. Behaviour 106: 43–62.

GREGORY, L.S. 1988. Population characteristics of Dolly Varden in the Tiekel River, Alaska. Master's thesis, University of Alaska, Fairbanks, Alaska.

GROSS, M.R. 1984. Sunfish, salmon and the evolution of alternative reproductive strategies and tactics in fishes, p. 55–75. *In* R. Wooton and G. Potts [ed.] Fish reproduction, strategies and tactics. Academic Press, London.

GROSS, M.R. 1985. Disruptive selection for alternative life history in salmon. Nature (Lond.) 313: 47–48.

HANSON, A.J., AND H.D. SMITH. 1967. Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age-groups. J. Fish. Res. Board Can. 24: 1955–1977.

HINO, T., K. MAEKAWA, AND J.B. REYNOLDS. !990. Alternative male mating behaviours in landlocked Dolly Varden (*Salvelinus malma*) in south-central Alaska. J. Ethol. 8: 13–20.

JARVI, T. 1990. The effects of male dominace, secondary sexual characteristics and female mate choice on the mating success of male Atlantic salmon *Salmo salar*. Ethology 84: 123–132.

JONES, J.W. 1959. The salmon. Collins, London.

KEENLEYSIDE, M.H.A., AND H.M.C. DUPUIS. Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. 66: 262–265.

KITANO, S. 1992. Individual variation of spawning behaviour in male Dolly Varden, *Salvelinus malma*. Master's thesis, Hokkaido University, Sapporo, Japan. 33 p. (In Japanese)

MAEKAWA, K. 1977. Studies of the variability of the land-locked Miyabe charr, *Salvelinus malma miyabei* 1. Development and early life history. Jpn. J. Ecol. 27: 91–102. (In Japanese with English summary)

MAEKAWA, K. 1983. Streaking behaviour of mature male parrs of the Miyabe charr, *Salvelinus malma miyabei*, during spawning. Jpn. J. Ichthyol. 30: 227–234.

MAEKAWA, K. 1984. Life history patterns of Miyabe charr in Shikaribetsu Lake, Japan, p. 223–250. In L. Johnson and B.L. Burns [ed.] Biology of the arctic charr. University of Manitoba Press, Winnipeg, Man.

MAEKAWA, K., AND T. HINO. 1986. Spawning behaviour of Dolly Varden in southeastern Alaska, with special reference to the mature male parr. Jpn. J. Ichthyol. 32: 454–458.

MAEKAWA, K., AND T. HINO. 1987. Effect of cannibalism on alternative life histories in charr. Evolution 41: 1120–1123.

MAEKAWA, K., AND T. HINO. 1990. Spawning tactics of female Miyabe charr (*Salvelinus malma miyabei*) against cannibalism. Can. J. Zool. 68: 889–894.

MAEKAWA, K., AND H. ONOZATO. 1986. Reproductive tactics and fertilization success of mature male Miyabe charr, *Salvelinus malma miyabei*. Environ. Biol. Fishes 15: 119–129.

MAYNARD SMITH, J. 1982. Evolution and the theory of games. Cambridge University Press, Cambridge, U.K.

REYNOLDS, J.S., AND M.R. GROSS. 1990. Costs and benefits of female mate choice: is there a lek paradox? Am. Nat. 136: 230–243.

SCHRODER, S.L. 1981. The role of sexual selection in determining the overall mating pattern and mate choice in chum salmon. Ph.D. thesis, University of Washington, Seattle, Wash.

SIGURJONSDOTTIR, H., AND K. GUNNARSSON. 1989. Alternative mating tactics of arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. Environ. Biol. Fishes 24: 159–176.

VAN DEN BERGHE, E.P., F. WERNERUS, AND R.T. WARNER. 1989. Female choice and the mating cost of peripheral males. Anim. Behav. 38: 875–884.

# Predicting Microdistributions of Steelhead (*Oncorhynchus mykiss*) Parr from Depth and Velocity Preference Criteria: Test of an Assumption of the Instream Flow Incremental Methodology

Hal A. Beecher, Thom H. Johnson, and John P. Carleton

*Washington Department of Wildlife, 600 Capitol Way N., Olympia, WA 98501-1091, USA*

Beecher, H.A., T.H. Johnson, and J.P. Carleton. 1993. Predicting microdistributions of steelhead (*Oncorhynchus mykiss*) parr from depth and velocity preference criteria: test of an assumption of the Instream Flow Incremental Methodology. Can. J. Fish. Aquat. Sci. 50: 2380–2387.

We tested an assumption of the Physical Habitat Simulation of the Instream Flow Incremental Methodology (IFIM) that fish select microhabitats based on the quality of one or several hydraulic conditions. We developed preference curves for juvenile steelhead (*Oncorhynchus mykiss*) in Morse Creek, Washington, USA, that accounted for availability of depths and velocities and their utilization by steelhead parr. To allow comparison of intervals among preference curves from different studies, we developed preference indices. We then evaluated the relationship between steelhead parr density and preference or preference indices for depth, velocity, and depth and velocity combined using an independent data set from a different year and an adjacent location in Morse Creek; these indices reflected observed densities of steelhead parr. There was a significant rank correlation between steelhead parr density and preferences or preference indices of steelhead parr for velocity alone and for depth and velocity combined, but not for depth alone. Steelhead parr strongly avoided habitat in which depth preference was 0.0, but velocity preference appeared to influence use of habitat where depth preference was not 0.0. Steelhead parr avoided cells with low preference indices and preferred cells with high preference indices. These relationships support an assumption of the IFIM.

Nous avons vérifié une hypothèse de la simulation de l'habitat physique tirée du *Instream Flow Incremental Methodology* (IFIM) selon laquelle le poisson choisi des micro-habitats en fonction de la qualité d'un ou de plusieurs paramètres hydrauliques. Nous avons établi des courbes de préférence pour des juvéniles de truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) du ruisseau Morse, à Washington (É.-U) qui tenaient compte des profondeurs et des vitesses de l'eau et de leur utilisation par des tacons de cette espèce. Afin de pouvoir comparer les intervalles entre les courbes de préférence des différents études, nous avons établi des indices de préférence. Nous avons ensuite évalué le rapport entre la densité des tacons et la préférence ou les indices de préférence pour la profondeur, la vitesse, la profondeur et la vitesse combinées au moyen d'un ensemble de données indépendantes d'une autre année et d'un emplacement voisin dans le ruisseau Morse : ces indices correspondaient aux densités observées de tacons. Il existait une corrélation des rangs importante entre la densité des tacons et les préférences ou indices de préférence des tacons pour la vitesse seule et pour la profondeur et la vitesse combinées, mais aucune pour la profondeur seulement. Les tacons évitaient fortement l'habitat dans lequel la préférence de profondeur était de 0,0, mais la préférence de vitesse semblait influer sur l'utilisation de l'habitat lorsque la préférence de profondeur était différente de 0,0. Les tacons évitaient les cases présentant des indices de préférences faibles, et recherchaient des cases à indices élevés. Ces rapports renforcent une hypothèse de l'IFIM.

*Received July 12, 1991*
*Accepted May 31, 1993*
*(JB142)*

*Reçu le 12 juillet 1991*
*Accepté le 31 mai 1993*

The Instream Flow Incremental Methodology (IFIM) (Stalnaker 1980; Bovee 1982) is considered the state-of-the-art instream flow method (Loar et al. 1985; Reiser et al. 1989), although several recent reports (Mathur et al. 1985; Scott and Shirvell 1987) have been critical of it. A prominent part of IFIM is the Physical Habitat Simulation (PHABSIM), a set of computer models that can be used to model how some aspects of habitat vary with flow. To use PHABSIM, assumptions about the dynamic equilibrium of channel form and features, about hydraulics, about fish ecology and behavior, and about fish population dynamics are all required.

Among the assumptions in PHABSIM are that fish selectively occupy preferred habitats and that their habitat preferences are based predominantly on their preferences for depth, velocity, substrate, and cover (Bovee and Cochnauer 1977; Stalnaker 1980; Bovee 1986). A testable extension of this assumption is that if (1) the distributions of depths, velocities, substrates, and cover are known and (2) the relative preferences of fish for different values of these habitat components are known, then fish distribution within a stream should be predictable. This study addresses only depth and velocity distribution relative to fish distribution, so it is not a complete test of the assumption. We reasoned that if fish habitat preference were to be useful in predicting fish distribution, prediction should at least be successful under similar conditions (flow and season) to those when preference curves were developed; if not, then predictions at other flows would be questionable.

In this study, we determined depth and velocity preferences of

juvenile (parr) steelhead (*Oncorhynchus mykiss*) in a stream segment. For adjacent stream segments, we then predicted fish distribution based on those depth and velocity preferences and compared the predicted and observed fish distributions.

The standard application of PHABSIM calculates fish habitat preference as the product of preferences for depth, velocity, and substrate or cover. Alternative calculations of combined preference are available (Bovee 1982, 1986; Milhous et al. 1984, option 19 in table V.1; Bovee and Zuboy 1988; C. Stalnaker, U.S. Fish and Wildlife Service, personal communication). Some users of PHABSIM in Washington State have suggested that alternative calculations might have more biological significance than the standard calculation. Accordingly, we also evaluated several alternative calculations of fish habitat preference.

## Study Site

Our study site was located about 3 km upstream from the mouth of Morse Creek, near Port Angeles, on the north slope of the Olympic Mountains, Washington. Morse Creek is a small (mean annual flow 3.58 $m^3/s$, drainage area ~146 $km^2$, mainstem length 26.1 km) steep stream (average gradient >6%) that flows into the Juan de Fuca Strait within sight of its source in alpine snowfields. In preliminary sampling, we had learned that this study area had a high density of juvenile steelhead relative to other streams we have sampled in Washington state. Morse Creek is typical of many streams where PHABSIM has been used to determine instream flow requirements for hydroelectric projects.

Morse Creek is fed by extensive snowfields on the north side of Hurricane Ridge (near 1950 m above mean sea level) in Olympic National Park. Snowmelt peaks in June (median June flow = 5.5 $m^3/s$) and continues through July. Low flows occur from late August through October, depending on onset of autumn rains, with the lowest monthly median flow of 1.0 $m^3/s$ in September. Highest flows occur with heavy rains during November–January (Williams et al. 1985).

The study site consisted of three adjacent segments, each about 150 m long. In the middle segment, we measured depths and velocities available to and used by juvenile steelhead during September 1987 and developed preference curves. The middle segment consisted of two pools, up to 2 m deep with gravel and cobble substrate, separated by a bedrock chute. The right bank was a cutbank. The left bank was a gravel bar. We observed gradual transitions of pool, run, and riffle habitats.

We sampled two adjacent test segments immediately upstream and downstream, respectively, of the habitat preference curve development segment during September 1989 to compare the distribution and habitat preference of steelhead parr with the preference curves developed 2 yr previously. In the test segments the left banks were cutbanks and the right banks were gravel bars. Each test segment had a longer pool and a shorter pool with gravel and cobble substrate. One of the lower pools was about 2 m deep, but the upper pools did not exceed about 1.5 m during our sampling. In at least one pool in the upper and lower segments, there was a gradual transition into runs and riffles. The upper segment contained a small bedrock chute. All three segments contained some bedrock outcrops.

Flow at the study site on the date of preference curve development in 1987 was 0.86 $m^3/s$, and stream width averaged 12.6 m with a range from 7.4 to 25.2 m. On the date of our distribution test in 1989, flow was 0.69 $m^3/s$, and stream width averaged 11.4 m with a range from 5.8 to 17.5 m.

The stream is inhabited by steelhead, anadromous cutthroat trout (*Oncorhynchus clarki*), chinook (*O. tshawytscha*), coho (*O. kisutch*), chum (*O. keta*), and pink salmon (*O. gorbuscha*), and Dolly Varden (*Salvelinus malma*), as well as unidentified sculpins (*Cottus* sp.). Juvenile steelhead fry (~30–60 mm total length (TL)), and parr (~75–200 mm TL) and coho salmon (~50–100 mm TL) are numerically dominant, with both species occupying pools and steelhead predominating in riffles and runs.

## Methods

### Habitat Preference Curve Development

We developed habitat preference curves by measuring depths and velocities used by fish (utilization) and available to the fish (availability) and then calculated preference as a normalized ratio of utilization to availability for different intervals of depth and velocity.

On 3–4 September 1987, from midmorning until visibility declined with low sun angle in late afternoon, two observers snorkeled upstream through two pools, two bedrock chutes, and a riffle, marking positions of steelhead parr with weighted flags. They swam in parallel, covering the full width of the stream. Water clarity was sufficient that we could see clearly underwater across the stream. We marked fish positions only after we were certain the fish were not being disturbed, that is, they displayed behavior such as feeding, territorial defense, and returning to position after the position was marked.

After flags were placed, we measured depth and mean column velocity (velocity at 0.6 depth) and estimated substrate composition at each of 104 flags. We used a top-setting wading rod with a Swoffer propeller current meter calibrated to read depth and velocity in imperial units (feet and feet per second).

After marking fish positions and measuring habitat use through the entire stream segment, we determined depth and velocity availability by measuring depth and mean column velocity at 182 points at 1.5-m intervals along transects spaced 6.2 m apart along the stream. (In the downstream-most 7.7 m of the site, we measured availability along transects that were 1.5 m apart and adjusted availability calculations to account for the greater density of measurements in this area.)

We developed depth and velocity preferences, as well as an alternative preference index, from the 1987 data. We established initial intervals of depth and mean column velocity (hereafter, velocity) at 0.03-m and 3-cm/s intervals and then tabulated observed numbers of parr into these initial intervals. We tabulated depth or velocity availability measurements into the same initial intervals and then calculated what percentage of total depth or velocity availability occurred in each interval.

We calculated expected numbers of parr in each initial interval by multiplying total number of observed parr by the percentage of depth or velocity availability in an interval. As used throughout this paper, the term "expected number of parr" or "expected number" means the number of parr that should have occurred in an interval of depth or velocity if the parr had distributed themselves in proportion to the availability of the interval. We combined intervals until expected numbers of parr in each interval exceeded five, except that the final interval was not combined with the preceding interval if the expected value in the final interval exceeded two parr. We combined intervals to avoid dividing either by zero or by very small numbers in the next step.

To develop preference curves that account for distribution of

available habitat and its utilization by steelhead parr, we divided observed number of parr (Ob) by expected number of parr ($E$) for each interval. To generate a preference factor between 0.0 and 1.0, the Ob/$E$ ratio in each interval was normalized by dividing each Ob/$E$ ratio by the maximum Ob/$E$ ratio. That is, $P = (\text{Ob}/E) / (\text{Ob}/E_{max})$. In this way, the most preferred interval had a preference of 1.0, and less preferred intervals had preferences between 0.0 and 1.0. Preferences were calculated for depth ($P[d]$) and velocity ($P[v]$) and then preference for depth and velocity combined was calculated as $P[dv] = P[d] \times P[v]$.

To minimize the subjectivity in fitting data to a curve, we did not smooth the preference curves. In most studies, preference curves are smoothed to follow a continuous distribution assumed to reflect fish behavior.

To allow comparison of intervals among preference curves from different studies, we developed a preference index. A preference index (PI) is developed from Ob/$E$ ratios in intervals before they are normalized into a preference curve. We think of where Ob/$E$ = 1 as a threshold between avoidance and preference. Ob/$E$ = 1 where fish occur in a depth, velocity, or depth–velocity interval in direct proportion to the area of stream channel available in the interval. Ob/$E$ < 1 where fish occur less often than expected based on availability of a habitat interval and suggests habitat avoidance, while Ob/$E$ > 1 where fish occur more often than expected and suggests habitat preference. To develop a preference index, Ob/$E$ ratios in intervals are divided into sections where Ob/$E$ < 1 and Ob/$E$ > 1. We assigned PI = 0.5 as the threshold or midpoint of the depth preference index (PI[$d$]) and velocity preference index (PI[$v$]). To generate a PI factor between 0 and 1, preference indices for depth or velocity below the threshold were calculated as $\text{PI} = 0.5 \times \text{Ob}/E$, and preference indices above the threshold were calculated as $\text{PI} = 0.5 + 0.5 [([\text{Ob}/E] - 1) / ([\text{Ob}/E_{max}] - 1)]$. In this way, PI < 0.5 in depth or velocity intervals avoided by fish and PI > 0.5 in depth or velocity intervals preferred by fish. The preference index for depth and velocity combined is calculated as $\text{PI}[dv] = \text{PI}[d] \times \text{PI}[v]$, and the PI[$dv$] threshold is the product of the PI[$d$] and PI[$v$] thresholds, i.e., $0.5 \times 0.5 = 0.25$.

If no interval had Ob/$E$ = 1 exactly, we found the interval with the Ob/$E$ ratio closest to 1.0 and averaged the midpoints of the adjacent intervals above and below that interval to calculate the threshold. The threshold can occur in an interval with a normalized preference ($P$) anywhere between 0.0 and 1.0.

### Predicting Steelhead Parr Distribution

In two adjacent sections of Morse Creek in a different year (1989), we measured depth and velocity distribution, predicted the distribution of steelhead parr using the preference curves we developed in Morse Creek 2 yr previously, and then measured steelhead parr distribution to test the predictions. We reasoned that steelhead parr density would be higher where fish habitat preferences were higher and that density would be lower where habitat preferences were lower.

On 27 September 1989, an observer counted parr by snorkeling along transects in the test segments that were adjacent upstream and downstream to the preference curve development segment. Parr were counted in the immediate vicinity (within 1.23 m of transect) of all points on a randomly placed grid in the stream; we then measured depths and velocities at those points. We calculated steelhead parr preferences for those points using preference factors determined in 1987 and discussed in the Preference Curve Development portion of this paper.

In the morning, we established transects across the stream at 15.4-m intervals along a line extending downstream from the lower end of the 1987 study reach. In the afternoon, we established transects across the stream at 6.2-m intervals along a line extending upstream from the upper end of the 1987 study reach because we had missed many concentrations of fish using 15.4-m intervals. Our starting transects were set at random distances along the line. We progressed upstream until underwater visibility declined with low sun angle in late afternoon.

At each transect, we visually identified the opposite end; then, one observer entered the water downstream from one end of the transect, snorkeled slowly up to the end of the transect, and then snorkeled across it, marking with weighted flags the positions of any steelhead parr that appeared to be within 1.23 m of the transect. (To be certain that we recorded data on all fish that were within 1.23 m of the transect, we marked some fish positions farther than 1.23 m from the transect, but later discarded those observations when distance from the transect was determined.)

After marking positions of any steelhead parr near a transect, we measured depth and velocity at 0.9-m intervals along the transect, beginning from the upstream/downstream line, regardless of the distance to water's edge from the line. We measured the distance from each marker (parr position) to the transect and used the nearest measurements of depth and velocity on the transect to represent the cell containing the parr.

The sampling procedure used in September 1989 yielded a series of cells along each transect. Each measurement point on a transect was the center of two cells, one rectangular ($0.9 \times 2.46$ m) and one square ($0.9 \times 0.9$ m); the larger rectangular cell encompassed the smaller square cell. Consistent with PHABSIM, depth and velocity at the measurement point were assumed to be representative of depth and velocity throughout the cell. This assumption was probably more true for smaller square cells and less true for the larger rectangular cells, as more points in the larger cells were farther from the measurement point. By using the larger rectangular cells, however, we doubled the number of fish and cells occupied by fish, and thus provided more powerful tests at the greater risk that some fish in a cell occupied depths and velocities that differed substantially from those at the measurement point for the cell.

We transcribed data on location, depth, velocity, preferences, and preference indices for each cell, and number of parr within square cells and within rectangular cells to a database. The steelhead parr preference factors and preference index factors associated with a cell were based on preference factors and preference index factors calculated during the development of the preference curves and preference indices for steelhead parr in Morse Creek during 1987. Each cell was thus associated with three preference factors ($P[d]$, $P[v]$, $P[dv]$) and three preference index factors (PI[$d$], PI[$v$], PI[$dv$]). The preference factors and preference index factors were sorted by value and then combined into intervals to bring the expected number of parr in an interval to at least five and as close to five as possible in the final interval. In this section of the study, combined preference intervals are generally broader than the combined intervals during preference curve development because, while the range of habitat measurements was about the same in the two portions of the study, we observed 50 parr during this portion compared with 104 parr during preference curve development.

The preference for a preference interval was calculated as the sum of the products of each preference or preference index and

TABLE 1. Depth and velocity preferences ($P$) and preference indices (PI) for steelhead parr in Morse Creek, September 1987.

| Depth ($d$) interval (m) | Proportion of total area available in interval | Number of parr | | | | | Velocity ($v$) interval (cm/s) | Proportion of total area available in interval | Number of parr | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Observed (Ob) | Expected ($E$) | Ob/$E$ | $P[d]$ | PI[$d$] | | | Observed (Ob) | Expected ($E$) | Ob/$E$ | $P[v]$ | PI[$v$] |
| <0.15 | 0.35 | 0 | 36.4 | 0.00 | 0.00 | 0.00 | <3.0 | 0.24 | 9 | 25.2 | 0.36 | 0.18 | 0.18 |
| 0.15–0.18 | 0.10 | 2 | 10.7 | 0.19 | 0.04 | 0.10 | 3.0–8.8 | 0.10 | 7 | 9.7 | 0.72 | 0.36 | 0.36 |
| 0.18–0.24 | 0.10 | 4 | 10.0 | 0.40 | 0.08 | 0.20 | 9.1–11.9 | 0.07 | 5 | 7.4 | 0.68 | 0.34 | 0.34 |
| 0.24–0.27 | 0.08 | 14 | 8.3 | 1.68 | 0.35 | 0.59 | 12.2–14.9 | 0.05 | 1 | 5.4 | 0.19 | 0.09 | 0.10 |
| 0.27–0.30 | 0.07 | 6 | 7.3 | 0.82 | 0.17 | 0.41 | 15.2–21.0 | 0.08 | 9 | 8.2 | 1.09 | 0.55 | 0.55 |
| 0.30–0.33 | 0.05 | 13 | 5.2 | 2.50 | 0.53 | 0.70 | 21.3–27.1 | 0.05 | 10 | 5.0 | 1.98 | 1.00 | 1.00 |
| 0.34–0.39 | 0.07 | 8 | 7.7 | 1.04 | 0.22 | 0.51 | 27.4–33.2 | 0.08 | 15 | 7.9 | 1.90 | 0.96 | 0.96 |
| 0.40–0.48 | 0.05 | 12 | 5.5 | 2.18 | 0.46 | 0.66 | 33.5–39.3 | 0.06 | 8 | 6.0 | 1.32 | 0.67 | 0.66 |
| 0.49–0.76 | 0.09 | 26 | 9.3 | 2.78 | 0.59 | 0.74 | 39.6–45.4 | 0.07 | 11 | 6.9 | 1.60 | 0.81 | 0.81 |
| >0.76 | 0.04 | 19 | 4.0 | 4.74 | 1.00 | 1.00 | 45.7–51.5 | 0.06 | 10 | 5.7 | 1.75 | 0.88 | 0.88 |
| | | | | | | | 51.8–60.7 | 0.06 | 9 | 7.0 | 1.28 | 0.65 | 0.64 |
| | | | | | | | 61.0–106.4 | 0.08 | 9 | 7.4 | 1.22 | 0.62 | 0.61 |
| | | | | | | | >106.4 | 0.02 | 1 | 2.0 | 0.50 | 0.25 | 0.25 |

the proportion of the combined preference interval area made up by each component preference interval. For example, a combined preference interval covering 0.2–0.3 might be made up of two component preference intervals, one at 0.2 with three as the expected number of parr and the other at 0.3 with two as the expected number of parr. The preference for the combined 0.2–0.3 preference interval would be $(3/5 \times 0.2) + (2/5 \times 0.3) = 0.24$.

In each combined preference interval, we calculated parr density as number of parr observed divided by the number of cells in that interval.

Our null hypothesis was that there was no significant relationship between rank of parr density and rank of preferences ($P[d]$, $P[v]$, $P[dv]$) or between rank of parr density and rank of preference indices (PI[$d$], PI[$v$], PI[$dv$]) for that combined preference interval. We considered rank correlations significant where $p < 0.05$ and marginally significant where $p < 0.10$. We used Kendall's tau to test for rank correlation because the data did not appear to be normally distributed.

A second null hypothesis was that parr would be distributed above or below the avoidance–preference threshold in proportion to the number of measurements (cells) in a preference interval and independent of preference index (PI[$d$], PI[$v$] or PI[$dv$]). For each preference interval, we calculated the expected number of parr within 0.45 m and within 1.23 m by multiplying total number of parr observed by the proportion of total number of measurements that were within that preference interval. We used chi-square tests of distribution and considered chi-square values significant where $p < 0.05$ and marginally significant where $p < 0.10$.

## Results

### Habitat Preference Curves

We measured depths and velocities at locations of 104 steelhead parr in Morse Creek during September 1987. Most parr were at locations with depths greater than 0.24 m and with velocities between 15 and 106 cm/s (Table 1). When habitat availability was considered, steelhead parr preferred (i.e., Ob/$E$ ratio > 1) these same intervals of depth and velocity, except that the depth interval 0.27–0.30 m was not preferred by parr (Table 1). The most-used interval differed from the most-preferred interval for both depth and velocity. The highest numbers of parr in an interval were observed at locations with depths of 0.49–0.76 m, but the depths most preferred by parr (i.e., having the highest Ob/$E$ ratio) were in the >0.76 m depth interval. Similarly, more parr were observed at locations with velocities of 27.4–33.2 cm/s, but the velocities most preferred by parr were in the 21.3–27.1 cm/s velocity interval. In addition, steelhead parr avoided (i.e., Ob/$E$ ratio < 1) the most available depths (those <0.24 m) and velocities (those <21 cm/s), the shallowest depths and slowest velocities available (Table 1). Depth and velocity preference curves for steelhead parr in Morse Creek are shown in Fig. 1.

For 179 locations on transects in Morse Creek, we measured a maximum depth of 1.2 m and a maximum velocity of 160 cm/s. We observed steelhead parr at a maximum depth of 1.7 m and at a maximum velocity of 139 cm/s. Maximum depth at locations of steelhead parr in Morse Creek was probably limited by stream size and maximum depth available at the flow during the study (0.86 $m^3/s$). Maximum velocity at locations of steelhead parr was less likely a function of maximum velocity available, as indicated by the declining Ob/$E$ ratio at higher velocities (Table 1).

### Predicting Steelhead Parr Distribution

On 27 September 1989, we observed and marked locations of 50 parr in 40 rectangular (2.4 × 0.9 m) cells. This included 25 parr in 21 square (0.9 × 0.9 m) cells.

Depth and velocity are significant factors influencing the spatial distribution of steelhead parr in Morse Creek during late summer low flow. Steelhead parr avoided cells with low preference indices and occurred in greater numbers than expected in cells with high preference indices. When we compared preference indices less than the threshold of 0.5 (PI values < 0.5) with preference indices greater than the threshold of 0.5 (PI values > 0.5), steelhead parr occurred significantly less frequently than expected in square and rectangular cells with PI[$v$] < 0.5 and significantly less frequently than expected in rectangular cells with PI[$d$] < 0.5. Similarly, steelhead parr preferred square and rectangular cells with PI[$v$] > 0.5 and preferred rectangular cells with PI[$d$] > 0.5 (chi-square tests, $p < 0.05$). Although parr occurred less frequently than expected in square cells with PI[$d$] < 0.5, the relationship was not significant (Table 2). When depth

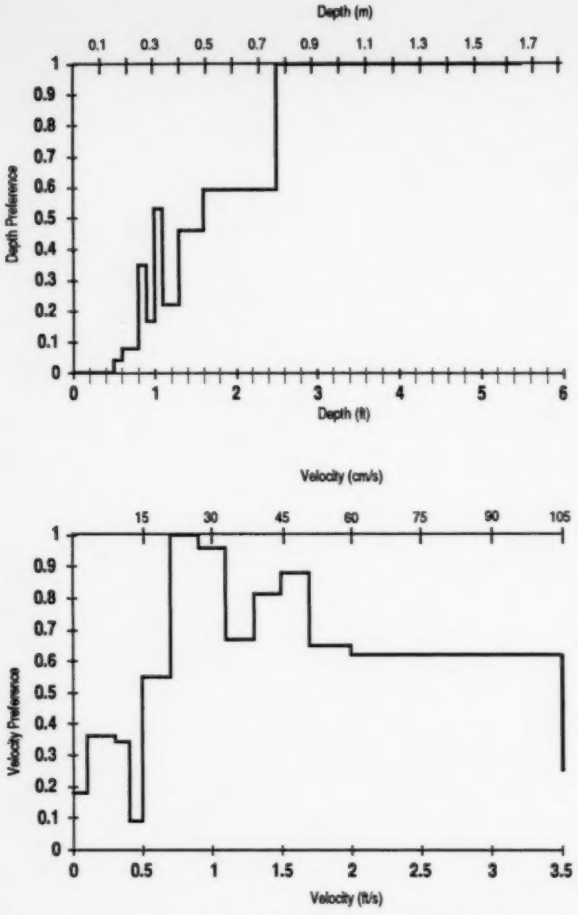



FIG. 1. Depth (upper panel) and velocity (lower panel) preference curves for steelhead parr in Morse Creek, September 1987. Preference curves were not smoothed.

and velocity are combined, the appropriate threshold between avoidance and preference is $0.5 \times 0.5 = 0.25$; steelhead parr occurred significantly less frequently than expected in square and rectangular cells having PI[$dv$] < 0.25, and parr preferred cells having PI[$dv$] > 0.25 ($p < 0.01$, Table 2).

Depth and velocity, alone and in combination, are important in determining steelhead parr distribution in Morse Creek. Depth appears to influence steelhead parr distribution mostly in areas with a depth preference of 0.0. A large number of cells had $P[d] = 0.0$ (Table 1) and parr avoided these cells ($p < 0.01$, Table 2). In areas where $P[d] > 0.0$, velocity appears to influence steelhead parr distribution more than depth. Comparisons of the significance levels of preferences and preference indices for depth and velocity ($P[d]$ versus $P[v]$ and PI[$d$] versus PI[$v$]) suggest that velocity provides a better measure of steelhead parr distribution in rectangular cells (Table 3).

Habitat preference and preference indices reflected observed densities of steelhead parr in Morse Creek. There was a significant relationship between steelhead parr density and preferences or preference indices for velocity alone and for depth and velocity combined, but not for depth alone. Rank correlation between steelhead parr habitat preferences and observed parr density in cells was significant for $P[dv]$ and PI[$dv$] in rectangular cells ($p < 0.05$) and marginally significant for $P[v]$ and PI[$v$] in rectangular cells and for $P[dv]$ and PI[$dv$] in square cells ($p < 0.10$), but relationships between $P[d]$ or PI[$d$] and parr density were not significant (Table 3). For example, parr density in an interval increased consistently with $P[dv]$ and PI[$dv$] in rectangular cells (Table 4; Fig. 2).

## Discussion

### Habitat Preference Curves

We accounted for availability of different intervals of depth and velocity and their use by steelhead parr when developing preference curves. Although depth and velocity preferences are probably not perceived independently by fish (Mathur et al. 1985; Scott and Shirvell 1987), we chose to treat them independently because it is the standard practice in PHABSIM studies in Washington State, and this study was designed as an initial test of the validity of studies using this approach.

We evaluated mean column velocity as a determinant of steelhead parr distribution because it is the standard method in PHABSIM studies in Washington. Scott and Shirvell (1987) criticized that the use of mean column velocity measured at 0.6 depth was not applicable to fish habitat selection. Shirvell (1989) improved the PHABSIM prediction of chinook salmon spawning locations by using focal point velocity rather than mean column velocity. Baltz et al. (1987, 1991) found that mean column velocity and focal point velocity were highly correlated in California streams. A high correlation ($r = 0.877$, $n = 435$) was found between mean column velocity and focal point velocity for spawning sockeye salmon (*Oncorhynchus nerka*) in Washington (M. Barclay and P. Rittmueller, Cascades Environmental Services, 1111 N. Forest St., Bellingham, Wash., personal communication).

Standard application of PHABSIM includes measurements of substrate or cover in addition to depth and velocity. We did not include substrate and cover but do not believe this seriously compromised the study because substrate and cover did not vary greatly throughout the study reaches. Substrate was relatively uniform with poorly sorted cobble and gravel throughout the study reach, but some boulders and bedrock were present. Only a few larger trout (not steelhead parr) and char were associated with bedrock outcrops, and we estimated that large boulders accounted for less than 1% of the study reach. Cobble, which was widely distributed, provided the main cover. Instream woody debris was absent in this segment of Morse Creek.

Sample size affects resolution of habitat preference curves. Small numbers of fish observations necessitate wider intervals within which to accumulate both observations and expected numbers of observations. As intervals widen, the chance increases that true preferences at the extremes and the midpoint of the range will differ. For example, the depth interval of 0.49–0.76 m was calculated to have a preference of 0.59 (see Table 1), but true depth preference might be greater at 0.76 m and less at 0.49 m or vice versa.

Preference curves developed in this study showed some anomalies that are probably artifacts of small sample size. Depth and velocity preference curves each have more than one peak, while one would expect unimodal curves. If deviations of our preference curves from the true preferences of steelhead parr and our small sample size made our test less likely to detect a

TABLE 2. Chi-square analyses of steelhead parr distribution among cells categorized as avoided (PI < 0.5) or preferred (PI > 0.5) and among cells having depth preferences ($P[d]$) of 0.0 or greater than 0.0.

| | Square cells (0.9 × 0.9 m) | | | Rectangular cells (0.9 × 2.4 m) | | |
|---|---|---|---|---|---|---|
| | Number of parr | | | Number of parr | | |
| Preference index | Observed (Obs) | Expected ($E$) | Cell chi-square | Observed (Ob) | Expected ($E$) | Cell chi-square |
| PI[$d$] < 0.5 | 14 | 16.53 | 0.39 | 23 | 33.06 | 3.06 |
| PI[$d$] > 0.5 | 11 | 8.47 | 0.76 | 27 | 16.94 | 5.98 |
| | | Chi-square = 1.15 | | | Chi-square = 9.04 | |
| | | df = 1, NS | | | df = 1, $p < 0.01$ | |
| PI[$v$] < 0.5 | 3 | 10.89 | 5.71 | 7 | 21.77 | 10.02 |
| PI[$v$] > 0.5 | 22 | 14.11 | 4.41 | 43 | 28.23 | 7.73 |
| | | Chi-square = 10.12 | | | Chi-square = 17.76 | |
| | | df = 1, $p < 0.01$ | | | df = 1, $p < 0.01$ | |
| PI[$dv$] < 0.25 | 10 | 17.74 | 3.38 | 20 | 35.48 | 18.30 |
| PI[$dv$] > 0.25 | 15 | 7.26 | 8.26 | 30 | 14.52 | 4.97 |
| | | Chi-square = 11.64 | | | Chi-square = 23.27 | |
| | | df = 1, $p < 0.01$ | | | df = 1, $p < 0.01$ | |
| $P[d] = 0.0$ | 3 | 9.48 | 4.43 | 7 | 18.95 | 7.54 |
| $P[d] > 0.0$ | 22 | 15.52 | 2.70 | 43 | 31.05 | 4.60 |
| | | Chi-square = 7.13 | | | Chi-square = 12.14 | |
| | | df = 1, $p < 0.01$ | | | df = 1, $p < 0.01$ | |

TABLE 3. Relationship between steelhead parr density and preference ($P$) and preference indices (PI) for depth ($d$), velocity ($v$), and depth–velocity ($dv$) combined.

| | $P[d]$ | $P[v]$ | $P[dv]$ | PI[$d$] | PI[$v$] | PI[$dv$] |
|---|---|---|---|---|---|---|
| | | | *Parr in square cells (0.9 × 0.9 m)* | | | |
| Kendall's tau | 0.33 | 0.67 | 1.00 | 0.33 | 0.67 | 1.00 |
| $n$ | 4 | 4 | 4 | 4 | 4 | 4 |
| $p$ | NS | NS | 0.10 | NS | NS | 0.10 |
| | | | *Parr in rectangular cells (0.9 × 2.4 m)* | | | |
| Kendall's tau | 0.60 | 0.73 | 0.97 | 0.33 | 0.73 | 0.81 |
| $n$ | 5 | 6 | 6 | 5 | 6 | 7 |
| $p$ | NS | 0.10 | 0.05 | NS | 0.10 | 0.05 |

TABLE 4. Rank of steelhead parr density, rank of depth–velocity preference ($P[dv]$), and rank of depth–velocity preference index (PI[$dv$]) in rectangular cells (0.9 × 2.4 m) as examples of the data used to test the relationships shown in Table 3.

| $P[dv]$ | | | Parr density | | $P[dv]$ | | | Parr density | |
|---|---|---|---|---|---|---|---|---|---|
| Interval | Weighted mean of interval | Rank | Parr per cell | Rank | Interval | Weighted mean of interval | Rank | Parr per cell | Rank |
| 0.00 | 0.00 | 6 | 0.07 | 6 | 0.00 | 0.00 | 7 | 0.07 | 7 |
| 0.01–0.03 | 0.02 | 5 | 0.10 | 5 | 0.02–0.06 | 0.04 | 6 | 0.15 | 5 |
| 0.04–0.07 | 0.05 | 4 | 0.24 | 3.5 | 0.07–0.12 | 0.10 | 5 | 0.12 | 6 |
| 0.08–0.14 | 0.10 | 3 | 0.24 | 3.5 | 0.13–0.19 | 0.16 | 4 | 0.23 | 4 |
| 0.15–0.29 | 0.21 | 2 | 0.26 | 2 | 0.20–0.36 | 0.30 | 3 | 0.27 | 3 |
| 0.30–0.59 | 0.42 | 1 | 0.50 | 1 | 0.38–0.51 | 0.45 | 2 | 0.46 | 1 |
| | | | | | >0.51 | 0.63 | 1 | 0.43 | 2 |
| Kendall's tau = 0.97, $n = 6$, $p < 0.05$ | | | | | Kendall's tau = 0.81, $n = 7$, $p < 0.05$ | | | | |

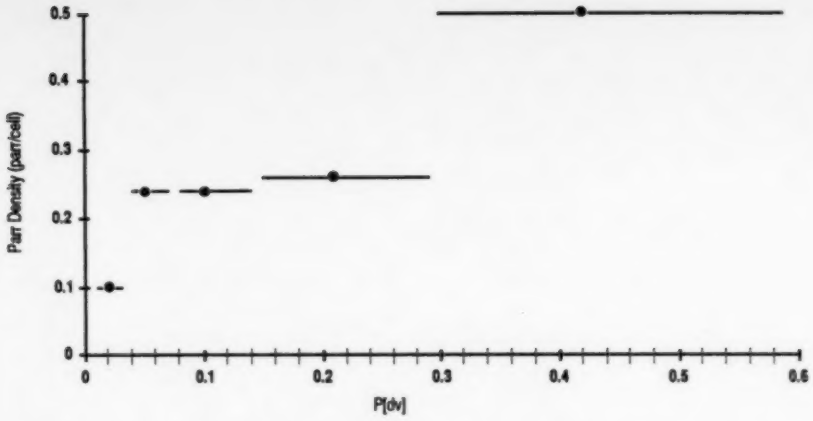



FIG. 2. Relationship between steelhead parr density and depth–velocity preference ($P[dv]$) intervals (horizontal lines) in Morse Creek from the data in Table 4. Circles indicate weighted means of $P[dv]$) intervals.

relationship between preference factors and fish distribution, the fact that we still found one suggests that the relationship is relatively robust.

## Predicting Steelhead Parr Distribution

Steelhead parr distribution during late summer low flow in Morse Creek was consistent with an assumption used in PHABSIM that depth and velocity preferences govern the usability and the use of habitat. Out of 12 analyses, the highest rank correlations were between density and the combined preference factor ($P[dv]$) and combined preference index (PI[$dv$]) (Table 3). We believe that the higher significance for $P[dv]$ and PI[$dv$] indicates that preference for depth and velocity combined is a better predictor of steelhead parr habitat preference than the other predictors we evaluated.

In this study, depth and velocity preferences were determined in the same stream as the test of predictions of fish distribution. The test of depth and velocity as predictors of habitat use was statistically independent of preference curve development, both spatially and temporally, but conditions were very similar (adjacent parts of same stream with similar habitat at similar streamflow).

Hardy et al. (1982) found positive correlations between predicted and observed distributions of native and introduced fishes in a spring-fed Nevada stream. Their predictions were based on depth and velocity only. Hardy et al. (1982) noted that cover distribution appeared to account for discrepancies between observed and predicted fish distributions. In a Michigan stream, Gowan (1984) found more brown trout (*Salmo trutta*) near transects with higher average weighted usable area (WUA, based on preferences for depth, velocity, and substrate) than in transects with lower average WUA. Scott and Shirvell (1987) questioned Gowan's conclusions because some trout occupied areas with preference values near zero, and some habitats with high preference values were not occupied, and further, Shirvell (1987) determined that the Michigan results were based on inappropriate data manipulation.

Shirvell (1989) tested the ability of PHABSIM to predict chinook salmon spawning distribution in a segment of the Nechaco River, British Columbia. In one of six simulations, he found a correlation coefficient of 0.80 between transect WUA and number of spawning salmon on the transect, but the same simulation incorrectly predicted that areas were unusable where over 50% of spawning had historically occurred.

In this study, as in those by Gowan (1984) and Shirvell (1989), we observed some fish in cells with preference factors of zero and some unoccupied cells that had high preference factors. Sampling artifacts during preference curve development and single-point representation of a cell may have contributed to this imprecision. In PHABSIM studies, measurement points on transects are assumed to be representative of a cell; however, streams are not homogeneous and few cells are uniform in depth, velocity, and substrate. A measurement at the center of a cell may indicate a preference of zero, but part of the cell might have a higher "true" preference and could possibly account for the presence of fish in a cell for which the preference is counted as zero. Similarly, most of a cell might have a true preference of zero, while the measurement at the center indicates a nonzero preference. In addition, the seeding rate of a stream can affect utilization of habitat by fish. Wild steelhead spawner escapement in Morse Creek that produced the parr observed in 1987 and 1989 met the escapement goal.

## Alternative Habitat Preference Index (PI)

We tested an alternative habitat preference index (PI) to accommodate some PHABSIM users' suggestion that a "neutral" preference value of 0.5 should be comparable between studies and should be a dividing point between preferred habitat ($Ob/E > 1$) and avoided habitat ($Ob/E < 1$) (Bozek and Rahel 1991). If PIs at a given depth or velocity or substrate class in separate studies are all above 0.5, then they are all preferred habitat; if they are all below 0.5, then they are all avoided habitat.

In our study, PI was about as useful as the standard preference factor ($P$) in predicting steelhead parr distribution. When PIs for depth, velocity, and substrate or cover are multiplied together in the standard application of PHABSIM, it should be noted that the combined "neutral" PI is 0.125.

## Assumptions of PHABSIM

Basic assumptions in IFIM are that (1) distribution of depth and velocity in relation to somewhat fixed channel features (substrate, cover) contributes to determining the quality of different areas or microhabitats to fish (Stalnaker 1980; Bovee 1982;

Milhous et al. 1984), (2) fish select microhabitats based on quality as determined by depth, velocity, substrate, and cover (possibly in conjunction with other features) (Stalnaker 1980; Bovee 1982; Milhous et al. 1984), (3) changes in flow directly change the quality of different microhabitats by changing depth and velocity distribution, while fish preferences for these attributes remain unchanged (Stalnaker 1980; Bovee 1982), and (4) fish populations are influenced by the history of the quality of the habitats they experience through one or more generations (Bovee 1982, 1988).

This study, together with studies by Hardy et al. (1982) and perhaps Gowan (1984), supports basic assumption (2) above. Shirvell's (1989) correlation between transect suitability and salmon redds near transects also provides limited support for this assumption, but PHABSIM's calculation of habitat suitability for chinook salmon spawning does not adequately predict spawning sites, thus casting doubt on this assumption for spawning habitat. Although none of these studies found perfect matches between fish use and indices of habitat quality, the number of positive associations suggests that this assumption is reasonable.

One of the secondary assumptions of a standard application of PHABSIM is that cell preference is a product of separate preferences for depth, velocity, and substrate. Shirvell (1989) speculated that his simulations of salmon spawning habitat may have been inaccurate because other hydraulic or topographic features were not incorporated into the calculation of WUA. This study supports reliance on calculation of combined preference as a product of separate preferences for depth and velocity. We also found depth and velocity preferences to be useful alone. Substrate measurements were not made in this study.

## Acknowledgments

We thank France Hoang, Mary Ellen Voss, and Darrell Pruett for preparing figures. Dr. Cole S. Shirvell (Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C.) and Dr. Donald J. Orth and Mr. Matthew J. Sabo (Virginia Polytechnic Institute and State University, Blacksburg, Va.) reviewed drafts of the manuscript and provided many helpful insights, comments, and suggestions.

## References

BALTZ, D.M., B. VONDRACEK, L.R. BROWN, AND P.B. MOYLE. 1987. Influence of temperature on microhabitat choice by fishes in a California stream. Trans. Am. Fish. Soc. 116: 12–20.

BALTZ, D.M., B. VONDRACEK, L.R. BROWN, AND P.B. MOYLE. 1991. Seasonal changes in microhabitat selection by rainbow trout in a small stream. Trans. Am. Fish. Soc. 120: 166–176.

BOVEE, K.D. 1982. A guide to stream habitat analysis using the Instream Flow Incremental Methodology. Instream Flow Information Paper 12. U.S. Fish Wildl. Serv. FWS/OBS-82-26: 248 p.

BOVEE, K.D. 1986. Development and evaluation of habitat suitability criteria for use in the Instream Flow Incremental Methodology. Instream Flow Information Paper 21. U.S. Fish Wildl. Serv. Biol. Rep. 86(7): 235 p.

BOVEE, K.D. 1988. Use of the instream flow incremental methodology to evaluate influences of microhabitat variability on trout populations in four Colorado streams. Proc. 68th Annu. Conf. Western Assoc. Fish Wildl. Agencies: 227–257.

BOVEE, K.D., AND T. COCHNAUER. 1977. Development and evaluation of weighted criteria, probability-of-use curves for instream flow assessments: fisheries. Instream Flow Information Paper No. 3. U.S. Fish Wildl. Serv. FWS/OBS-77/63: 39 p.

BOVEE, K., AND J.R. ZUBOY [ED.] 1988. Proceedings of a workshop on the development and evaluation of habitat suitability criteria. U.S. Fish Wildl. Serv. Biol. Rep. 88(11): 407 p.

BOZEK, M.A., AND F.J. RAHEL. 1991. Assessing habitat requirements of young Colorado River cutthroat trout using macrohabitat and microhabitat approaches. Trans. Am. Fish. Soc. 120: 571–581.

GOWAN, C. 1984. The impacts of irrigation water withdrawals on brown trout (*Salmo trutta*) and two species of benthic macroinvertebrates in a typical southern Michigan stream. M.S. thesis, Department of Fisheries and Wildlife, Michigan State University, Lansing, Mich. 127 p.

HARDY, T.B., C.G. PREWITT, AND K.A. VOOS. 1982. Application of a physical habitat usability model to the fish community in a spring-fed desert stream, p. 391–397. *In* W.K. Lauenroth, G.V. Skogerboe, and M. Flug [ed.] Analysis of ecological systems: state-of-the-art in ecological modelling. Developments in environmental modelling 5. Elsevier Science Publishing Co., Inc., New York, N.Y.

LOAR, J.M., M.J. SALE, G.F. CADA, D.K. COX, R.M. CUSHMAN, G.K. EDDLEMON, J.L. ELMORE, A.J. GATZ, P. KANCIRUK, J.A. SOLOMON, AND D.S. VAUGHAN. 1985. Application of habitat evaluation models in southern Appalachian trout streams. Oak Ridge Natl. Lab. Environ. Sci. Div. Publ. No. 2382, ORNL/TM-9323, Oak Ridge, Tenn. 310 p.

MATHUR, D., W.H. BASON, E.J. PURDY, JR., AND C.A. SILVER. 1985. A critique of the Instream Flow Incremental Methodology. Can. J. Fish. Aquat. Sci. 42: 825–831.

MILHOUS, R.T., D.L. WEGNER, AND T. WADDLE. 1984. User's guide to the Physical Habitat Simulation system (PHABSIM). Instream Flow Information Paper 11, U.S. Fish Wildl. Serv. FWS/OBS-81/43.

REISER, D.W., T.A. WESCHE, AND C. ESTES. 1989. Status of instream flow legislation and practices in North America. Fisheries 14(1): 22–29.

SCOTT, D., AND C.S. SHIRVELL. 1987. A critique of the Instream Flow Incremental Methodology and observations on flow determination in New Zealand, p. 27–44. *In* J.F. Craig and J.B. Kemper [ed.] Regulated streams: advances in ecology. Plenum Press, New York and London. 431 p.

SHIRVELL, C.S. 1987. Does the IFIM have biological significance: a critique. Instream Flow Chron. IV(2): 1, 3.

SHIRVELL, C.S. 1989. Ability of PHABSIM to predict chinook salmon spawning habitat. Regul. Rivers Res. & Manage. 3: 277–289.

STALNAKER, C.B. 1980. The use of habitat structure preferenda for establishing flow regimes necessary for maintenance of fish habitat, p. 321–337. *In* J.V. Ward and J.A. Stanford [ed.] The ecology of regulated streams. Plenum Press, New York and London.

WILLIAMS, J.R., H.E. PEARSON, AND J.D. WILSON. 1985. Streamflow statistics and drainage-basin characteristics for the Puget Sound region, Washington. Volume 1: Western and southern Puget Sound. U.S. Geol. Surv. Open-File Rep. 84–144-A, Tacoma, Wash.

# Adjusting Mercury Concentration for Fish-Size Covariation: A Multivariate Alternative to Bivariate Regression

Keith M. Somers[1] and Donald A. Jackson

*Department of Zoology, University of Toronto, Toronto, ON M5S 1A1, Canada*

Somers, K.M., and D.A. Jackson. 1993. Adjusting mercury concentration for fish-size covariation: a multivariate alternative to bivariate regression. Can. J. Fish. Aquat. Sci. 50: 2388–2396.

Regression-based methods like analysis of covariance (ANCOVA) are frequently used to adjust one variable for the correlated influence of a second less interesting variable (e.g., mercury concentration and fish size). However, the influence of the covariate (i.e., fish size) is not removed unequivocally when regression slopes are not parallel. Using data on tissue-mercury concentration and fish size from 30 populations of lake trout (*Salvelinus namaycush*), we show that data adjusted to a common size with bivariate regression can retain information associated with the original size differences. As an alternative, we use univariate and bivariate summary statistics from each population as raw data in a multivariate analysis to search for differences among populations. Ordination axes resulting from this analysis exhibited both small- and large-scale spatial autocorrelation. Localized spatial patterns probably reflect similar geochemical features of the watersheds of neighbouring lakes in small geographic areas. In contrast, regional spatial autocorrelation suggested broad-scale patterns that may implicate atmospheric inputs of mercury. As an extension of this multivariate approach, both regional and local patterns could be compared with environmental variables to reveal correlations that may suggest new cause-and-effect hypotheses.

Des méthodes fondées sur la régression comme l'analyse de la covariance sont souvent utilisées pour ajuster une variable pour tenir compte de l'influence corrélée d'une deuxième variable moins intéressante (p.ex., la concentration de mercure et la taille du poisson). Toutefois, l'influence de la covariable (c.-à.-d la taille du poisson) n'est pas supprimée de façon univoque lorsque les pentes de régression ne sont pas parallèles. À l'aide de données sur la concentration de mercure dans les tissus et la taille des poissons de 30 populations de touladi (*Salvelinus namaycush*), nous avons montré que les données ajustées à une taille courante au moyen de la régression à deux variables peuvent garder des informations associées aux différences de taille originales. Comme autre possibilité, nous utilisons les statistiques sommaires à une variable et à deux variables de chaque population comme données brutes dans le cadre d'une analyse à plusieurs variables afin de déceler des différences entre les populations. Les axes d'ordination obtenues à partir de cette analyse démontraient une autocorrélation spatiale sur une petite échelle et sur une grande échelle. Des configurations spatiales localisées reflètent probablement des caractères géochimiques similaires de bassins hydrographiques des lacs voisins dans de petites zones géographiques. Par contraste, une autocorrélation spatiale régionale semblait indiquer des configurations sur une vaste échelle susceptibles de comporter des apports atmosphériques de mercure. Pour élargir cette approche à plusieurs variables, des configurations régionales et locales pourraient être comparées à des variables environnementales afin de mettre à jour des corrélations qui peuvent indiquer de nouvelles hypothèses cause–effet.

*Received July 24, 1991*
*Accepted May 31, 1993*
*(JB158)*

*Reçu le 24 juillet 1991*
*Accepté le 31 mai 1993*

The proper method to correct or adjust one variable for relatively uninteresting variation in a second variable is a long-standing issue in biological studies (e.g., Atchley 1978; Smith 1984; Reist 1985, 1986; Packard and Boardman 1988). The use of indices or ratios was once widely accepted as one such adjustment (e.g., condition factor, gonadosomatic index (GSI); liver somatic index (LSI), and so on; see Leatherland and Sonstegard 1987; Munkittrick and Dixon 1988; Willis 1989). But the recognition of statistical problems with ratios (e.g., Pearson 1897; Chayes 1971; Atchley et al. 1976; Atchley and Anderson 1978; Kenney 1982; Phillips 1983; Gibson 1984; Packard and Boardman 1987, 1988) has prompted the evaluation and use of numerous alternatives (e.g., Corruccini 1977; Strauss 1985; Craig and Babaluk 1989; Cone 1989; dos Reis et al. 1990; Jackson et al. 1990; Murphy et al. 1990).

Many of these alternatives incorporate regression-based procedures (e.g., Atchley 1978; Smith 1984; Green 1986); however, interpretive problems arise when comparing populations if regression slopes differ (Thorpe 1976; Reist 1985). Frequently, such problems emerge in studies of contaminants in animal tissues where contaminant concentration increases with animal size (e.g., mercury in fish; Wren and MacCrimmon 1983; Rada et al. 1986; McMurtry et al. 1989; Wiener et al. 1990; Wren et al. 1991) or with lipid concentration (e.g., Roberts et al. 1977; Borgmann and Whittle 1983; Möller et al. 1983). At issue is how to remove the correlated influence of animal size or lipid concentration so differences in contaminant concentrations can be examined and better understood.

One common approach employs analysis of covariance (ANCOVA) to compare slopes and intercepts of several regres-

[1]Present address: Limnology Section, Water Resources Branch, Ontario Ministry of the Environment, P.O. Box 39, Dorset, ON P0A 1E0, Canada.

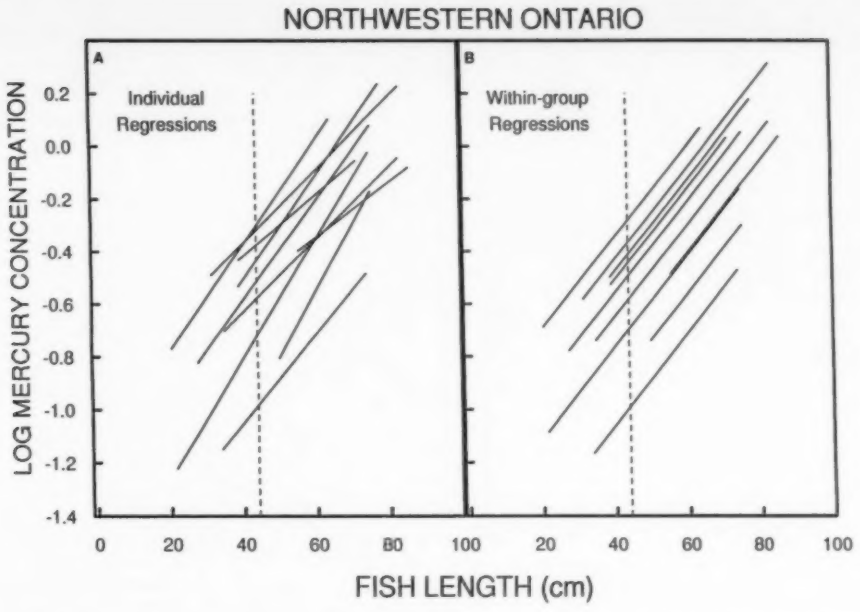



FIG. 1. Regressions of mercury concentration on fish length for 10 populations of lake trout from northwestern Ontario lakes. The adjusted mercury concentration for a standardized length of 44 cm is indicated (by the broken vertical line) for each population based on (A) individual regressions and (B) common within-groups regressions. Line lengths span the range of lengths in the collection for each population.

sions, thereby using bivariate regression to adjust for uncontrolled variation in the independent variable (i.e., $X$) (e.g., Green 1986; Packard and Boardman 1987, 1988; Tracy and Sugar 1989). An ANCOVA is relatively simple with a small number of regressions, but with large data sets and many regressions, the revelation that slopes differ leaves much to be desired. Differing slopes confound further analysis, much like a significant interaction between main effects in an analysis of variance (ANOVA) (see Sokal and Rohlf 1981, p. 414–417). As a result, the ANCOVA will not reliably evaluate whether intercepts differ (i.e., the usual test of interest) when regressions are not parallel. This limitation is particularly frustrating when the covarying $X$ variable could not be "controlled" at the time of data collection (e.g., Reist 1985; Packard and Boardman 1987; Meffe et al. 1988).

The problem of size adjustment only materializes when slopes are not parallel because ANCOVA automatically adjusts for the covariate and compares intercepts (or adjusted means) when the slopes are not different. However, when slopes differ, the common within-groups slope is recommended as the best estimate of the common slope (Thorpe 1976; Thorpe and Leamy 1983; Reist 1985) (e.g., see Baltz and Moyle 1981; Ihssen et al. 1981; Reist and Crossman 1987). This estimated slope is then used to calculate the intercept and adjusted mean concentation for each population, or the predicted concentration at some arbitrarily selected standard size. However, Reist (1985) cautioned that results from this type of adjustment may vary depending on the particular data set being analysed (e.g., see Thorpe 1976; Rayner 1985; Shea 1985).

In essence, the within-groups approach ignores the fact that slopes differ significantly among populations and simply estimates a common slope across all populations (see Fig. 1B). The suitability of this estimate depends on the relative variation of each population, since varying populations can have undue influence on the estimate of the common slope (e.g., see Thorpe 1976; Pimentel 1979, p. 81–91; Thorpe and Leamy 1983).

An alternative approach uses the individual regression equations to estimate mercury concentration at a common size for each population (see Fig. 1A) (e.g., Taylor and McPhail 1985; Johnson 1987; McMurtry et al. 1989). By using individual equations, significant differences in slopes are ignored although these differences are incorporated into the standardization. As a result, this method may combine differences in slopes with differences in intercepts (see below). The relative contributions of the slope and intercept depend on the scale of the original axes (e.g., the size of the slope relative to the intercept) and the common size selected. Because the choice to use the common, within-groups approach may also affect the results, we must choose between two potential distortions in order to examine other factors that correlate with observed differences among populations.

McMurtry et al. (1989) provided a recent example in an examination of the concentrations of mercury in dorsal muscle of populations of lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieu*) from approximately 90 lakes in Ontario. M.J. McMurtry (personal communication) first used ANCOVA to compare regressions of logarithmically transformed mercury concentration on fish length for each population because mercury concentration in fish muscle tends to covary with fish size (e.g., Scott and Armstrong 1972; Scott 1974; MacCrimmon et al. 1983; Sprenger et al. 1988). Unfortunately, the regression slopes differed among populations (e.g.,

see Fig. 1A). To explore environmental factors that correlated with these differences, McMurtry et al. (1989) subsequently chose to adjust observed mercury concentrations to a standard lake trout size of 44 cm using individual regression equations for each population (e.g., Fig. 1A). These adjusted values were then correlated with physical and chemical variables from each lake to determine which environmental factors best predicted size-adjusted tissue-mercury concentrations.

The estimated mercury concentration used by McMurtry et al. (1989) is a composite index based on the observed regression intercept plus the slope multiplied by the adjusted size (compare Fig. 1A and 1B). Although means and standard deviations of the original variables (i.e., fish length and mercury concentration) differed among populations, these differences were ignored because differences in the size distributions of the samples likely reflected collecting biases (M.J. McMurtry, personal communication).

By using either the within-groups or the individual regression adjustment, the bivariate relationships are reduced to a single index for each population (see Fig. 1). As a result, the means and standard deviations for each variable are combined with the regression slopes, intercepts, and correlations (i.e., weighted by the correlation; see Jensen 1986) to estimate a mercury concentration that should be independent of fish length. Unfortunately, this data-reduction approach pools information inconsistently because different weights are used for each population. As a result, similar size-adjusted values may arise even though the original values differed considerably. Henceforth, this difference is lost from subsequent analyses.

Alternatively, an analysis using univariate and bivariate summary statistics as new variables for each population may provide insight with less information loss (e.g., Atchley and Rutledge 1980; Rohlf and Archie 1984; Ferson et al. 1985). Because the summary statistics are estimated from the same variables, these statistics are not independent and hence, a multivariate analysis is warranted. If a priori groups or classes of populations exist (e.g., geographic regions), a multivariate analysis of variance (i.e., MANOVA) might be employed. Otherwise data exploration techniques such as principal components analysis would summarize covarying patterns among variables.

This paper focuses on the recurring problem of adjusting for a covariate when regression slopes differ (e.g., Whittle and Fitzsimons 1983; Johnson et al. 1987; Allard and Stokes 1989) by (1) illustrating the implications of such adjustments on analytical results and subsequent data interpretation, and (2) demonstrating an alternative multivariate approach to this problem. We use a fish-and-mercury example although this is a common problem relevant to physiology, morphometrics, and many other subdisciplines (e.g., see Reist 1985; Packard and Boardman 1987, 1988). To demonstrate the utility of our multivariate approach, we analyse data from 10 lake trout populations from each of three regions in Ontario to search for large-scale regional patterns as possible evidence of atmospheric deposition (i.e., spatial autocorrelation) (e.g., Jumars et al. 1977; Sokal and Oden 1978; Mackas 1984; Fortin et al. 1989).

Future studies should contrast these multivariate results with lake physical and chemical features to search for potential cause-and-effect relationships (as in McMurtry et al. 1989; Cope et al. 1990; Wiener et al. 1990). For example, spatial patterns in the multivariate results could be compared with patterns in the physical and chemical variables using Mantel tests (e.g., Jackson and Harvey 1989; Legendre and Fortin 1989) and Procrustes analysis (e.g., Digby and Kempton 1987, p. 112–123; Rising and Somers 1989; Rohlf and Slice 1990). Similarly, localized patterns could be explored with partial Mantel tests that first remove large-scale regional patterns (e.g., Hubert 1985; Manly 1986; Smouse et al. 1986; Bocquet-Appel and Sokal 1989).

## Methods

To explore this problem with a balanced design focusing on three geographic regions in Ontario, we selected 30 of the 91 regressions for lake trout populations that were described by McMurtry et al. (1989). Ten lakes were chosen from northwestern Ontario (i.e., with latitude >48° and longitude >89°), 10 from the Algoma area (i.e., 46–48° latitude and 82–85° longitude), and 10 from the Algonquin area (i.e., 44–46° latitude and 77–81° longitude) (see Table 1). Individual lakes were selected if at least 20 fish were sampled. Lakes with samples of at least 15 and 13 fish, respectively, were included to obtain 10 lakes from northwestern Ontario and the Algoma region.

Raw data for fish size and mercury concentration were summarized with the mean, standard deviation, minimum, and maximum to provide eight univariate summary variables (see Table 1). Regressions of the logarithm of mercury concentration in dorsal muscle and fish length (as in McMurtry et al. 1989) generated three bivariate statistics for each population (i.e., slope, intercept, and correlation). One-way ANOVAs were used to contrast these 11 variables from the three regions to assess geographic differences (SAS Institute Inc. 1988). A posteriori differences were identified with the least-significant-difference test.

The impact of subjectively choosing an adjusted size was explored by calculating predicted mercury concentration at fish lengths of 25, 44, 100, and 200 cm using individual population regressions, within-group regressions, and the common regression across all populations (i.e., approximately 0.5, 1, 2, and 4 times the value used by McMurtry et al. 1989). The resultant predicted mercury concentrations were correlated with the original population parameters using Spearman rank correlations.

Multivariate differences were assessed with a MANOVA and canonical variates analysis (CVA) (SAS Institute Inc. 1988). We then summarized patterns among the lakes with an ordination. The eight univariate and three bivariate summary statistics were each standardized to a 0–1 range to weight each variable equally without changing the distribution of the values. A Euclidean distance matrix was then calculated between the 30 lakes using these 11 standardized variables. This distance matrix was rotated to the first five principal coordinate axes with principal coordinates analysis (PCoA) (Gower 1966). Loadings of the 11 variables on these new axes were assessed with Spearman rank correlations because of nonnormality in the data. Spearman rank correlations were also calculated between the predicted-mercury variables and the PCoA axes.

To test for spatial autocorrelation, the Euclidean distance matrix based on the first five principal coordinates was compared with an interlake geographic distance matrix using a Mantel test and associated correlogram (e.g., see Sokal 1979; Legendre and Troussellier 1988; Bocquet-Appel and Sokal 1989; Legendre and Fortin 1989). We only used the first five PCoA axes to omit uncorrelated variation embedded in the entire data set (i.e., random noise) (see Gauch 1982). Both Pearson and Spearman correlations were evaluated in the Mantel tests (and associated

TABLE 1. Location, sample sizes, and univariate and bivariate summary statistics for 30 lake trout populations from three regions in Ontario.

| Region | | | Regression | | | Fish length (cm) | | | | Mercury concn. ($\mu g \cdot g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lake name | Lat., long. | $N$ | $r^2$ | Slope | Intercept | Mean | SD | Min. | Max. | Mean | SD | Min. | Max. |
| Northwestern Ontario | | | | | | | | | | | | | |
| Bending | 49°19′, 92°08′ | 20 | 0.447 | 0.012 | −0.89 | 56.5 | 11.3 | 39.2 | 71.5 | 0.67 | 0.420 | 0.30 | 2.20 |
| Greenwater | 48°34′, 90°26′ | 24 | 0.759 | 0.023 | −1.71 | 48.2 | 13.6 | 21.6 | 74.9 | 0.34 | 0.388 | 0.05 | 2.00 |
| Pickerel | 48°37′, 91°19′ | 23 | 0.618 | 0.014 | −0.93 | 57.4 | 14.7 | 31.6 | 83.3 | 0.89 | 0.667 | 0.31 | 3.20 |
| Snook | 50°12′, 94°41′ | 23 | 0.721 | 0.020 | −1.18 | 51.0 | 13.4 | 20.5 | 64.2 | 0.86 | 0.581 | 0.12 | 2.70 |
| Basswood | 48°05′, 91°35′ | 18 | 0.772 | 0.014 | −1.18 | 45.4 | 11.2 | 35.0 | 83.0 | 0.31 | 0.202 | 0.20 | 1.05 |
| Clearwater | 49°00′, 91°57′ | 17 | 0.728 | 0.025 | −2.06 | 59.0 | 7.4 | 50.0 | 75.4 | 0.29 | 0.134 | 0.10 | 0.60 |
| Paguchi | 49°34′, 91°32′ | 16 | 0.752 | 0.017 | −1.71 | 58.5 | 11.4 | 34.0 | 74.0 | 0.21 | 0.108 | 0.08 | 0.43 |
| Quetico | 48°34′, 91°55′ | 17 | 0.820 | 0.020 | −1.30 | 54.3 | 9.7 | 39.0 | 78.0 | 0.67 | 0.430 | 0.30 | 1.91 |
| Sandy Beach | 49°49′, 92°21′ | 16 | 0.276 | 0.011 | −0.98 | 68.6 | 8.2 | 55.6 | 85.8 | 0.58 | 0.214 | 0.27 | 0.98 |
| Trout | 50°14′, 94°55′ | 16 | 0.844 | 0.019 | −1.34 | 57.3 | 12.6 | 27.6 | 75.5 | 0.65 | 0.414 | 0.18 | 1.60 |
| Algoma | | | | | | | | | | | | | |
| Burns | 46°36′, 83°07′ | 59 | 0.838 | 0.031 | −2.37 | 34.1 | 11.6 | 20.2 | 75.0 | 0.08 | 0.090 | 0.01 | 0.50 |
| Esten | 46°22′, 82°40′ | 20 | 0.939 | 0.024 | −1.75 | 46.0 | 19.4 | 20.6 | 87.0 | 0.38 | 0.440 | 0.04 | 1.40 |
| Wawa | 48°01′, 84°43′ | 37 | 0.765 | 0.014 | −1.34 | 34.0 | 16.3 | 13.6 | 67.9 | 0.16 | 0.087 | 0.03 | 0.39 |
| Cobre | 46°38′, 82°48′ | 15 | 0.411 | 0.013 | −1.53 | 43.3 | 8.1 | 28.5 | 52.9 | 0.12 | 0.054 | 0.07 | 0.25 |
| Como | 47°55′, 83°30′ | 15 | 0.567 | 0.021 | −1.39 | 68.2 | 9.6 | 53.0 | 89.5 | 1.29 | 0.615 | 0.32 | 2.21 |
| Flack | 46°35′, 82°47′ | 17 | 0.257 | 0.013 | −1.31 | 40.1 | 5.0 | 29.0 | 48.0 | 0.17 | 0.046 | 0.10 | 0.25 |
| Lac Aux Sables | 46°47′, 82°20′ | 17 | 0.801 | 0.019 | −1.42 | 51.2 | 10.3 | 39.6 | 74.5 | 0.41 | 0.264 | 0.20 | 1.19 |
| Matinenda | 46°22′, 82°57′ | 15 | 0.880 | 0.020 | −1.52 | 48.7 | 13.7 | 19.5 | 68.4 | 0.34 | 0.231 | 0.11 | 0.81 |
| North Hubert | 47°20′, 84°27′ | 17 | 0.443 | 0.018 | −1.54 | 46.8 | 10.9 | 31.0 | 64.5 | 0.24 | 0.168 | 0.08 | 0.60 |
| Saymo | 46°59′, 83°31′ | 13 | 0.856 | 0.018 | −1.19 | 44.0 | 11.6 | 30.6 | 69.4 | 0.45 | 0.299 | 0.20 | 1.14 |
| Algonquin | | | | | | | | | | | | | |
| Bark | 45°27′, 77°51′ | 20 | 0.464 | 0.015 | −0.81 | 66.2 | 6.2 | 52.3 | 79.1 | 1.61 | 0.658 | 1.10 | 3.50 |
| Big Porcupine | 45°27′, 78°37′ | 24 | 0.751 | 0.037 | −2.18 | 36.2 | 6.5 | 25.5 | 45.3 | 0.18 | 0.102 | 0.05 | 0.39 |
| Bonnechere | 45°28′, 78°35′ | 26 | 0.787 | 0.026 | −1.61 | 40.7 | 8.2 | 21.2 | 54.1 | 0.33 | 0.177 | 0.12 | 0.69 |
| Burn's | 45°19′, 77°05′ | 29 | 0.254 | 0.019 | −1.81 | 30.5 | 5.4 | 23.0 | 43.0 | 0.06 | 0.024 | 0.01 | 0.13 |
| Camp | 45°26′, 78°55′ | 21 | 0.672 | 0.018 | −1.49 | 34.4 | 10.1 | 26.0 | 59.0 | 0.15 | 0.084 | 0.04 | 0.40 |
| Drag | 45°05′, 78°24′ | 35 | 0.663 | 0.025 | −1.69 | 38.1 | 11.0 | 24.0 | 74.0 | 0.27 | 0.330 | 0.04 | 1.50 |
| Fletcher | 45°21′, 78°47′ | 21 | 0.851 | 0.021 | −1.64 | 41.1 | 16.1 | 19.5 | 69.0 | 0.25 | 0.220 | 0.05 | 0.71 |
| Gull | 44°51′, 78°47′ | 20 | 0.366 | 0.010 | −0.98 | 31.7 | 5.4 | 21.0 | 40.2 | 0.23 | 0.050 | 0.15 | 0.36 |
| Haliburton | 45°12′, 78°24′ | 20 | 0.524 | 0.017 | −1.00 | 60.5 | 8.1 | 39.5 | 73.8 | 1.07 | 0.426 | 0.43 | 2.01 |
| Kennisis | 45°13′, 78°38′ | 20 | 0.850 | 0.024 | −1.82 | 41.7 | 10.2 | 21.4 | 66.9 | 0.19 | 0.198 | 0.07 | 0.99 |

correlograms) using two-tailed probabilities and 10 000 randomizations (as recommended by Jackson and Somers 1989; also see Legendre and Fortin 1989).

## Results

One-way ANOVAs of the raw data confirmed concerns expressed by McMurtry et al. (1989). The mean length of lake trout collected from northwestern Ontario lakes was significantly greater than the mean for lake trout collected from Algoma and Algonquin lakes ($F = 5.29$ with 2, 27 df, $P < 0.012$, $r^2 = 0.282$). In addition, the maximum length of lake trout differed among the three areas ($F = 4.77$ with 2, 27 df, $P < 0.017$, $r^2 = 0.261$). Maximum fish sizes from northwestern Ontario lakes were significantly larger than the Algonquin samples, and the Algoma fish were intermediate. None of the other summary statistics differed significantly among the regions ($P > 0.1$).

Regressions of the logarithm of mercury concentration and fish length displayed significantly different slopes across the 30 populations (ANCOVA, $F = 4.55$ with 29, 620 df, $P < 0.001$; with a common within-group regression slope of 0.0201). Significant heterogeneity of variance was also evident ($F_{max} = 8.93$ with 15, 18 df, $P < 0.001$). In addition, regression slopes were significantly different within regions (i.e., northwestern Ontario: $F = 2.33$ with 9, 179 df, $P < 0.025$, see Fig. 1A; $F_{max} = 4.15$ with 22, 16 df, $P < 0.005$; Algoma: $F = 7.93$ with 9, 214 df, $P < 0.001$; $F_{max} = 6.41$ with 15, 11 df, $P < 0.005$; Algonquin: $F = 2.69$ with 9, 225 df, $P < 0.01$; $F_{max} = 7.18$ with 33, 18 df, $P < 0.005$). The common, within-group regression slope was largest for the set of Algonquin lakes (0.0222) and decreased for the Algoma (0.0209) and northwestern Ontario lakes (0.0173).

Predicted mercury concentrations at lengths of 25, 44, 100, and 200 cm showed no significant differences among the regions (ANOVA, $P > 0.2$). Mercury values at 25 and 44 cm that were estimated with the individual regressions were significantly correlated with mean fish length, mean mercury concentration, and the regression intercept ($P < 0.05$) (Table 2). Mercury concentrations at a 25-cm length were also correlated with the regression slope. Correlations with mean fish length suggest that the adjusted values for fish 25 or 44 cm in length based on the individual regressions failed to remove the influence of fish size. In contrast, predicted mercury concentrations at 100 and 200 cm were correlated with the regression slope and correlation, but not mean size. Adjusted mercury at 100 cm was also correlated with mean mercury concentration whereas the 200-cm mercury concentration was correlated with the regression intercept.

These findings changed when using the common-slope adjustment or the comon, within-groups regression adjustment

TABLE 2. Spearman rank correlations (×1000) between common within-group-adjusted and individual-regression-adjusted mercury concentrations and univariate, bivariate, and multivariate summaries for lake trout populations from 30 Ontario lakes ($r_s = 0.375$ at $P < 0.05$, 0.483 at $P < 0.01$, 0.598 at $P < 0.001$).

| Regression adjustment and associated size for [Hg] prediction | Spearman rank correlation (×1000) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fish length | Mean Hg concn. | Regression | | | Principal coordinate axis | | | | |
| | | | Slope | Intercept | Correlation | I | II | III | IV | V |
| *Common slope across groups* | | | | | | | | | | |
| [Hg] at 44 cm | 305 | 758 | −178 | 742 | 002 | 576 | 013 | 325 | 380 | 395 |
| *Common within-group slope* | | | | | | | | | | |
| [Hg] at 25 cm | 439 | 832 | −256 | 810 | −051 | 694 | 029 | 291 | 285 | 315 |
| [Hg] at 44 cm | 325 | 766 | −213 | 776 | −024 | 591 | 031 | 318 | 360 | 388 |
| [Hg] at 100 cm | 053 | 524 | 000 | 508 | −018 | 289 | 056 | 231 | 540 | 377 |
| [Hg] at 200 cm | −272 | 104 | 101 | 156 | −013 | −113 | 110 | 135 | 509 | 325 |
| *Individual regression slope and intercept* | | | | | | | | | | |
| [Hg] at 25 cm | 453 | 767 | −538 | 956 | −224 | 687 | 242 | 347 | 114 | 315 |
| [Hg] at 44 cm | 451 | 840 | −173 | 747 | −040 | 683 | 042 | 202 | 345 | 301 |
| [Hg] at 100 cm | 096 | 425 | 730 | −147 | 394 | 246 | −497 | −278 | 654 | 046 |
| [Hg] at 200 cm | −047 | 089 | 974 | −606 | 567 | −039 | −650 | −407 | 449 | −083 |

TABLE 3. Spearman rank correlations (×1000) between univariate and bivariate summary statistics and the first five principal coordinate axes for lake trout populations from 30 Ontario lakes ($r_s = 0.375$ at $P < 0.05$, 0.483 at $P < 0.01$, 0.598 at $P < 0.001$). Dashes within the body of the table represent correlations between a variable and itself.

| Univariate and bivariate summary statistics | Spearman rank correlation (×1000) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fish length | Mean Hg concn. | Regression | | | Principal coordinate axis | | | | |
| | | | Slope | Intercept | Correlation | I | II | III | IV | V |
| Mean length | — | 798 | −151 | 392 | −052 | 893 | 075 | −313 | −218 | 021 |
| Length SD | 053 | 159 | 185 | −063 | 645 | 182 | −814 | 429 | −366 | −150 |
| Min. length | 722 | 494 | −339 | 397 | −410 | 639 | 557 | −494 | −172 | 094 |
| Max. length | 690 | 619 | 069 | 181 | 262 | 757 | −305 | −285 | −301 | −073 |
| Mean [Hg] | 798 | — | −078 | 620 | 074 | 937 | −093 | 021 | 101 | 139 |
| [Hg] SD | 686 | 917 | 136 | 415 | 250 | 883 | −379 | 061 | 146 | −026 |
| Min [Hg] | 742 | 834 | −396 | 769 | −171 | 802 | 318 | −081 | 036 | 380 |
| Max [Hg] | 678 | 906 | 095 | 461 | 181 | 891 | −322 | 046 | 200 | −007 |
| Slope | −151 | −078 | — | −744 | 565 | −186 | −647 | −422 | 384 | −141 |
| Intercept | 392 | 620 | −744 | — | −346 | 593 | 378 | 434 | −036 | 305 |
| Correlation | −052 | 074 | 565 | −346 | — | −002 | −846 | 022 | −150 | 382 |

(Table 2). Correlations between predicted mercury concentration using the within-groups adjustment and mean fish length or mean mercury concentration resembled correlations based on the individual-regression adjustment. However, correlations with the regression statistics differed (i.e., depending on the adjusted size), with no significant correlations with the regression slope or associated correlation coefficient. Clearly, adjusting data to a common size affects subsequent results and interpretations when regression slopes differ.

A PCoA of the univariate and bivariate statistics summarized 95% of the standardized variation in the first five axes (Table 3). The first axis accounted for 50% of the variation and included seven of the univariate variables (excluding the standard deviation of fish length) and the regression intercept. The second PCoA axis contrasted the standard deviation of length, plus the regression slope and correlation with minimum fish length and intercept (i.e., 24.6% of the variation).

One-way ANOVAs of the five sets of PCoA scores indicated that the three regions differed on the first axis ($F = 2.64$ with 2, 27 df, $P < 0.09$, $r^2 = 0.164$) and differed significantly on the fourth axis ($F = 5.98$ with 2, 27 df, $P < 0.008$, $r^2 = 0.307$). This fourth axis contrasted the maximum and standard deviation of fish length with the regression slope (Table 3; i.e., 7.9% of the variation). The Algonquin lakes had greater mean scores compared with the Algoma and northwestern Ontario lakes.

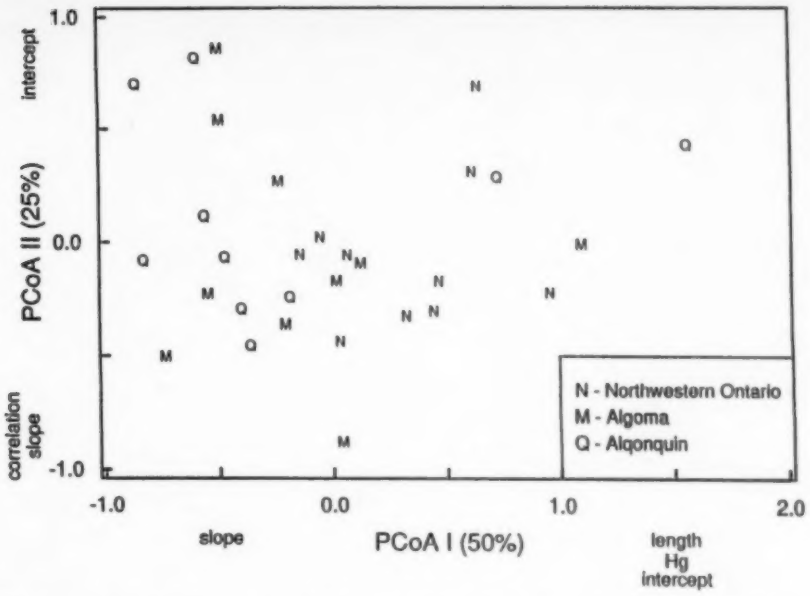



FIG. 2. Scatterplot of the first and second PCoA axes derived from the analysis of Euclidean distances among 30 lakes based on eight univariate and three bivariate summary statistics from data on lake trout length and tissue-mercury concentrations.

A MANOVA confirmed the univariate findings by revealing significant differences among the regions using the 11 variables (Wilk's lambda = 0.165, $F = 2.25$ with 22, 34 df, $P < 0.017$; Roy's Greatest Root = 2.98, $F = 4.87$ with 11, 18 df, $P < 0.002$). The CVA (not shown) emphasized that the multivariate differences arose because fish from northwestern Ontario were larger than the Algoma or Algonquin samples. A MANOVA using the five PCoA axes was also significant (Wilk's lambda = 0.490, $F = 1.97$ with 10, 46 df, $P < 0.059$; Roy's Greatest Root = 0.868, $F = 4.16$ with 5, 24 df, $P < 0.008$), emphasizing that the standardization and the choice to use only the information in the first five axes reduced the multivariate separation among the three regions.

The three sets of lakes overlap in the space defined by the first two PCoA axes (Fig. 2). Most of the northwestern Ontario lakes lie on the right side of the ordination, although this space is shared with four Algoma and two Algonquin lakes. These populations are characterized by larger fish with higher mercury concentrations and larger regression intercepts (Table 3). The remaining Algoma and Algonquin populations are intermixed, suggesting similarities among these populations with respect to the mercury and fish-length data.

The MANOVA based on the ordination revealed that populations of lake trout from the northwestern Ontario lakes show modest separation from the other populations (see Fig. 2). The overlap among lakes from the various regions suggests that factors contributing to differences in tissue mercury vary within a region (e.g., mean and maximum fish length). However, this observation does not discount a local-effects hypothesis that populations from nearby lakes (e.g., within the same watershed) exhibit similar mercury fish-size relationships.

The Mantel test indicated a significant correlation between distances among lakes on the first five PCoA axes and the geographic distances separating those same lakes ($r = 0.116$, $P < 0.017$; $r_s = 0.155$, $P < 0.001$). The larger rank correlation suggests that the relationship between the two distance matrices is curvilinear, with differing local and regional effects.

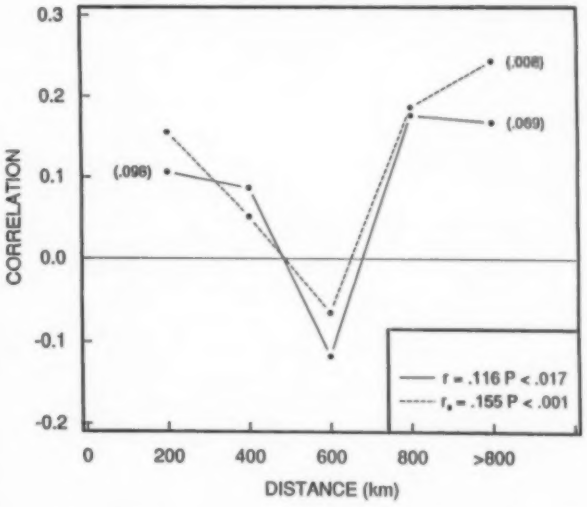



FIG. 3. Mantel test correlogram contrasting interlake distances from the first five PCoA axes and geographical distances. Overall Pearson and Spearman correlations are presented in the legend whereas class values are plotted (along with probabilities when less than 0.1). Class sample sizes are 115, 70, 66, 64 and 120, respectively. Distances represent class marks, not midpoints.

A correlogram showed that positive spatial autocorrelation exists among the nearest lakes, as well as the most distant lakes (Fig. 3). This observation suggests that mercury fish-size

relationships in lake trout populations in neighbouring lakes are similar ($r_s = 0.155$, $P < 0.098$; i.e., supporting the local-effects hypothesis). However, the strongest association existed between the most distant lakes (i.e., between regions; $r_s = 0.243$, $P < 0.008$), indicating significant large-scale regional patterns (i.e., the weak geographic clusters observed in Fig. 2). The shape of this correlogram is characteristic of a surface with a central peak or depression (e.g., see Legendre and Fortin 1989), although this pattern incorporates covariation associated with differences in fish length (i.e., length-related information in PCoA axis I that varies across regions; see Table 3).

A Mantel test and associated correlogram based on a distance matrix using the second through fifth PCoA axes omits a multivariate analogue of these length-correlated effects (e.g., see Somers 1989) and examines the spatial pattern of this four-dimensional configuration (i.e., 46.1% of the standardized variation in the original 11 variables). The overall Mantel test was not significant ($r = 0.023$, $P < 0.637$; $r_s = 0.051$, $P < 0.299$), emphasizing that the large-scale regional pattern was mainly based on the fish-size information in the first PCoA axis (see Table 3). The correlogram (not shown) was similar to the one based on all five axes (Fig. 3), with both small-scale (i.e., <200 km: $r_s = 0.164$, $P < 0.085$) and large-scale spatial autocorrelation approaching significance (600 km: $r_s = 0.213$, $P < 0.086$; 800 km: $r_s = 0.232$, $P < 0.068$; >800 km: $r_s = 0.151$, $P < 0.098$). The MANOVA using these four ordination axes provided similar results (Wilk's lambda = 0.614, $F = 1.65$ with 8, 48 df, $P < 0.135$; Roy's Greatest Root = 0.625, $F = 3.90$ with 4, 25 df, $P < 0.014$), given that Roy's Greatest Root has greater power to detect a gradient than Wilk's lambda (Clarke and Green 1988). These findings suggest that both local and regional patterns remained once the dominant fish-size effects were removed (i.e., patterns within areas, as well as among areas).

## Discussion

McMurtry et al. (1989) assumed that their standardized mercury concentration represented a size-adjusted concentration for each lake trout population. Our analysis of a subset of their data substantiates this assumption (see Table 2). However, our size-adjusted data were also correlated with mean fish length and mean mercury concentration, suggesting that the standardization failed to remove the confounding influence of fish size. Had McMurtry et al. (1989) chosen an adjusted length of 25, 100, or 200 cm (recognizing that these lengths would be extrapolations beyond the data), their results would have probably differed. The choice of an alternative regression adjustment (e.g., the within-groups regression) would also affect their results.

Given these inconsistencies with size adjustments when slopes differ, we propose that univariate and bivariate summary statistics should be analysed with multivariate methods as an alternative to the use of a single adjusted value. Similar approaches are common in morphometrics (e.g., Atchley and Rutledge 1980), especially when using Fourier analysis methods (e.g., Rohlf and Archie 1984; Ferson et al. 1985). An ordination of univariate and regression statistics provides less ambiguous results and summarizes covarying patterns into uncorrelated axes for comparison with other suites of variables.

Since regressions are influenced by sample sizes, plus the standard deviations and ranges of the variables, the discovery of clusters of populations or outliers in the resultant ordination will assist in resolving differences among populations. The ordination in this example revealed patterns reflecting geographical proximity (i.e., spatial autocorrelation), perhaps due to both local watershed or lake basin geology, and large-scale patterns that might be attributable to atmospheric inputs (e.g., Rada et al. 1989; Mierle 1990). In addition, prey type can influence contaminant accumulation (e.g., MacCrimmon et al. 1983; Mathers and Johansen 1985; Cope et al. 1990), so biogeographical patterns associated with the presence or absence of particular prey species could also contribute to observed groupings of lakes (Rasmussen et al. 1990).

To extend the original study of McMurtry et al. (1989), the resultant ordination axes could be compared with patterns from ordinations of physical or chemical variables using a Mantel test or Procrustes analysis (e.g., Digby and Kempton 1987, p. 112–123; Jackson and Harvey 1989). Such methods have fewer restrictive assumptions than multiple regression, especially stepwise procedures (Reckhow et al. 1987; James and McCulloch 1990).

Perhaps one drawback to this approach is the difficulty in resolving or interpreting multivariate axes (James and McCulloch 1990), although the reification of such axes is now widely accepted (Pimentel 1979, p. 7; Reyment 1990). Given that these types of studies are inherently multivariate (i.e., based on several response variables), single summary indices (such as the adjusted mercury concentration described herein) are inappropriate and can contribute to misleading results and interpretations (e.g., see Jackson et al. 1990).

To summarize, we caution against the routine use of individual or common within-group regressions to adjust data to remove the influence of a predictor variable when regression slopes differ. These types of adjustments can produce misleading results reflecting differences in regression slopes rather than intercepts, or retaining information correlated with the original size variable. As an alternative, we illustrate that multivariate procedures can be used to examine univariate and bivariate summary statistics to resolve and interpret patterns with less information loss. In addition, the resultant ordination axes can be used to evaluate correlations with various environmental factors, including geographic location (i.e., spatial autocorrelation).

## Acknowledgments

We thank R.W. Bilyea, R.L. France, D.L. Fuller, H.H. Harvey, K.A. Keenleyside, M.J. McMurtry, M.S. Ridgway, W.A. Scheider, R. Snell, D.L. Wales, and C.D. Wren for discussions and helpful suggestions. H.H. Harvey supervised this work. D.L. Wales, from the Fisheries Branch of the Ontario Ministry of Natural Resources, kindly provided the raw data for the mercury fish-size relationships. Portions of this study were funded by an NSERC scholarship to D.A.J. and an NSERC operating grant to H.H. Harvey.

## References

ALLARD, M., AND P.M. STOKES. 1989. Mercury in crayfish species from thirteen Ontario lakes in relation to water chemistry and smallmouth bass (*Micropterus dolomieui*) mercury. Can. J. Fish. Aquat. Sci. 46: 1040–1046.

ATCHLEY, W.R. 1978. Ratios, regression intercepts, and the scaling of data. Syst. Zool. 27: 78–83.

ATCHLEY, W.R., AND D. ANDERSON. 1978. Ratios and the statistical analysis of biological data. Syst. Zool. 27: 71–78.

ATCHLEY, W.R., C.T. GASKINS, AND D. ANDERSON. 1976. Statistical properties of ratios: I. Empirical results. Syst. Zool. 25: 137–148.

ATCHLEY, W.R., AND J.J. RUTLEDGE. 1980. Genetic components of size and shape. I. Dynamics of components of phenotypic variability and covariability during ontogeny in the laboratory rat. Evolution 34: 1161–1173.

BALTZ, D.M., AND P.B. MOYLE. 1981. Morphometric analysis of tule perch (*Hysterocarpus traski*) populations in three isolated drainages. Copeia 1981: 305–311.

BOCQUET-APPEL, J.-P., AND R.R. SOKAL. 1989. Spatial autocorrelation analysis of trend residuals in biological data. Syst. Zool. 38: 333–341.

BORGMANN, U., AND D.M. WHITTLE. 1983. Particle-size-conversion efficiency and contaminant concentrations in Lake Ontario biota. Can. J. Fish. Aquat. Sci. 40: 328–336.

CHAYES, F. 1971. Ratio correlation. University of Chicago Press, Chicago, Ill. 99 p.

CLARKE, K.R., AND R.H. GREEN. 1988. Statistical design and analysis for a 'biological effects' study. Mar. Ecol. Prog. Ser. 46: 213–226.

CONE, R.S. 1989. The need to reconsider the use of condition indices in fishery science. Trans. Am. Fish. Soc. 118: 510–514.

COPE, W.G., J.G. WIENER, AND R.G. RADA. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: relation to lake characteristics. Environ. Toxicol. Chem. 9: 931–940.

CORRUCCINI, R.S. 1977. Correlation properties of morphometric ratios. Syst. Zool. 26: 211–214.

CRAIG, J.F., AND J.A. BABALUK. 1989. Relationship of condition of walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*) to water clarity, with special reference to Dauphin Lake, Manitoba. Can. J. Fish. Aquat. Sci. 46: 1581–1586.

DIGBY, P.G.D., AND R.A. KEMPTON. 1987. Multivariate analysis of ecological communities. Chapman and Hall, London. 206 p.

DOS REIS, S.F., L.M. PESSOA, AND R.E. STRAUSS. 1990. Application of size-free canonical discriminant analysis to studies of geographic differentiation. Rev. Bras. Genet. 13: 509–520.

FERSON, S., F.J. ROHLF, AND R.K. KOEHN. 1985. Measuring shape variation of two-dimensional outlines. Syst. Zool. 34: 59–68.

FORTIN, M.-J., P. DRAPEAU, AND P. LEGENDRE. 1989. Spatial autocorrelation and sampling design in plant ecology. Vegetatio 83: 209–222.

GAUCH, H.G. JR. 1982. Noise reduction by eigenvector ordination. Ecology 63: 1643–1649.

GIBSON, A.R. 1984. Multivariate analysis of lizard thermal behavior. Copeia 1984: 267–272.

GOWER, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338.

GREEN, R.H. 1986. Some applications of linear models for analysis of contaminants in aquatic biota, p. 231–245. *In* A.H. El-Shaarawi and R.E. Kwiatowski [ed.] Statistical aspects of water quality monitoring. Elsevier, New York, N.Y.

HUBERT, L.J. 1985. Combinatorial data analysis: association and partial association. Psychometrika 50: 449–467.

IHSSEN, P.E., D.O. EVANS, W.J. CHRISTIE, J.A. RECKAHN, AND R.L. DESJARDINE. 1981. Life history, morphology, and electrophoretic characteristics of five allopatric stocks of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes Region. Can. J. Fish. Aquat. Sci. 38: 1790–1807.

JACKSON, D.A., AND H.H. HARVEY. 1989. Biogeographic associations in fish assemblages: local versus regional processes. Ecology 70: 1472–1484.

JACKSON, D.A., H.H. HARVEY, AND K.M. SOMERS. 1990. Ratios in aquatic sciences: statistical shortcomings with mean depth and the morphoedaphic index. Can. J. Fish. Aquat. Sci. 47: 1788–1795.

JACKSON, D.A., AND K.M. SOMERS. 1989. Are probability estimates from the permutation model of Mantel's test stable? Can. J. Zool. 67: 766–769.

JAMES, F.C., AND C.E. MCCULLOCH. 1990. Multivariate analysis in ecology and systematics: panacea or Pandora's box? Annu. Rev. Ecol. Syst. 21: 129–166.

JENSEN, A.L. 1986. Functional regression and correlation analysis. Can. J. Fish. Aquat. Sci. 43: 1742–1745.

JOHNSON, M.G. 1987. Trace element loadings to sediments of fourteen Ontario lakes and correlations with concentrations in fish. Can. J. Fish. Aquat. Sci. 44: 3–13.

JOHNSON, M.G., J.R.M. KELSO, AND S.E. GEORGE. 1987. Loadings of organochlorine contaminants and trace elements to two Ontario lake systems and their concentrations in fish. Can. J. Fish. Aquat. Sci. 45(Suppl. 1): 170–178.

JUMARS, P.A., D. THISTLE, AND M.L. JONES. 1977. Detecting two-dimensional spatial structure in biological data. Oecologia 28: 109–123.

KENNEY, B.C. 1982. Beware of spurious correlations! Water Resour. Res. 18: 1041–1048.

LEATHERLAND, J.F., AND R.A. SONSTEGARD. 1987. Comparative fecundity and age survival in two stocks of goitred coho salmon (*Oncorhynchus kisutch* Walbaum) from Lake Erie. Can. J. Zool. 65: 2780–2785.

LEGENDRE, P., AND M.-J. FORTIN. 1989. Spatial pattern and ecological analysis. Vegetatio 80: 107–138.

LEGENDRE, P., AND M. TROUSSELLIER. 1988. Aquatic heterotrophic bacteria: modelling in the presence of spatial autocorrelation. Limnol. Oceanogr. 33: 1055–1067.

MACCRIMMON, H.R., C.D. WREN, AND B.L. GOTS. 1983. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 40: 114–120.

MACKAS, D.L. 1984. Spatial autocorrelation of plankton community composition in a continental shelf ecosystem. Limnol. Oceanogr. 29: 451–471.

MANLY, B.F.J. 1986. Randomization and regression methods for testing associations with geographical, environmental and biological distances between populations. Res. Popul. Ecol. 28: 201–218.

MATHERS, R.A., AND P.H. JOHANSEN. 1985. The effects of feeding ecology on mercury accumulation in walleye (*Stizostedion vitreum*) and pike (*Esox lucius*) in Lake Simcoe. Can. J. Zool. 63: 2006–2012.

MCMURTRY, M.J., D.L. WALES, W.A. SCHEIDER, G.L. BEGGS, AND P.E. DIMOND. 1989. Relationship of mercury concentrations in lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) to the physical and chemical characteristics of Ontario lakes. Can. J. Fish. Aquat. Sci. 46: 426–434.

MEFFE, G.K., D.L. CERTAIN, AND A.L. SHELDON. 1988. Selective mortality of post-spawning yellowfin shiners, *Notropis lutipinnis* (Pisces: Cyprinidae). Copeia 1988: 853–858.

MIERLE, G. 1990. Aqueous inputs of mercury to Precambrian Shield lakes in Ontario. Environ. Toxicol. Chem. 9: 843–851.

MÖLLER, H., R. SCHNEIDER, AND C. SCHNIER. 1983. Trace metal and PCB content of mussels (*Mytilus edulis*) from the southwestern Baltic Sea. Int. Rev. Gesamten Hydrobiol. 68: 633–647.

MUNKITTRICK, K.R., AND D.G. DIXON. 1988. Growth, fecundity, and energy stores of white sucker (*Catostomus commersoni*) from lakes containing elevated levels of copper and zinc. Can. J. Fish. Aquat. Sci. 45: 1355–1365.

MURPHY, B.R., M.L. BROWN, AND T.A. SPRINGER. 1990. Evaluation of the relative weight (Wr) index, with new applications to walleye. N. Am. J. Fish. Manage. 10: 85–97.

PACKARD, G.C., AND T.J. BOARDMAN. 1987. The misuse of ratios to scale physiological data that vary allometrically with body size, p. 216–239. *In* M.E. Feder, A.F. Bennett, W.W. Burggren, and R.B. Huey [ed.] New directions in ecological physiology. Cambridge University Press, New York, N.Y.

PACKARD, G.C., AND T.J. BOARDMAN. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiol. Zool. 61: 1–9.

PEARSON, K. 1897. On a form of spurious correlation which may arise when indices are used in the measurement of organs. Proc. R. Soc. Lond. 60: 489–502.

PHILLIPS, R.B. 1983. Shape characters in numerical taxonomy and problems with ratios. Taxon 32: 535–544.

PIMENTEL, R.A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall/Hunt Publ. Co., Dubuque, Iowa. 276 p.

RADA, R.G., J.E. FINDLEY, AND J.G. WIENER. 1986. Environmental fate of mercury discharged into the Upper Wisconsin River. Water Air Soil. Pollut. 29: 57–76.

RADA, R.G., J.G. WIENER, M.R. WINFREY, AND D.E. POWELL. 1989. Recent increases in atmospheric deposition of mercury to north-central Wisconsin lakes inferred from sediment analyses. Arch. Environ. Contam. Toxicol. 18: 175–181.

RASMUSSEN, J.B., D.J. ROWAN, D.R.S. LEAN, AND J.H. CAREY. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. 47: 2030–2038.

RAYNER, J.M.V. 1985. Linear relations in biomechanics: the statistics of scaling functions. J. Zool. (Lond.) 206: 415–439.

RECKHOW, K.H., R.W. BLACK, T.B. STOCKTON, JR., J.D. VOGT, AND J.G. WOOD. 1987. Empirical models of fish response to lake acidification. Can. J. Fish. Aquat. Sci. 44: 1432–1442.

REIST, J.D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can. J. Zool. 63: 1429–1439.

REIST, J.D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Can. J. Zool. 64: 1363–1368.

REIST, J.D., AND E.J. CROSSMAN. 1987. Genetic basis of variation in morphometric characters as implied by hybrids between subspecies of *Esox americanus* (Pisces: Esocidae). Can. J. Zool. 65: 1224–1229.

REYMENT, R.A. 1990. Reification of classical multivariate statistical analysis in morphometry, p. 123–144. *In* F.J. Rohlf and F.L. Bookstein [ed.] Proc. Michigan Morphometrics Workshop. Spec. Publ. No. 2, Museum of Zoology, University of Michigan, Ann Arbor, Mich.

RISING, J.D., AND K.M. SOMERS. 1989. The measurement of overall body size in birds. Auk 106: 666–674.

ROBERTS, J.R., A.S.W. DEFRIETAS, AND M.A.J. GIDNEY. 1977. Influence of lipid pool size on bioaccumulation of the insecticide chlordane by northern redhorse suckers (*Moxostoma macrolepidotum*). J. Fish. Res. Board Can. 34: 89–97.

ROHLF, F.J., AND J. ARCHIE. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Syst. Zool. 33: 302–317.

ROHLF, F.J., AND D. SLICE. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Zool. 39: 40–59.

SAS INSTITUTE INC. 1988. SAS/STAT user's guide, 6.03 edition. Cary, N.C. 1028 p.

SCOTT, D.P. 1974. Mercury concentration of white muscle in relation to age, growth, and condition in four species of fishes from Clay Lake, Ontario. J. Fish. Res. Board Can. 31: 1723–1729.

SCOTT, D.P., AND F.A.J. ARMSTRONG. 1972. Mercury concentration in relation to size in several species of freshwater fishes from Manitoba and northwestern Ontario. J. Fish. Res. Board Can. 29: 1685–1690.

SHEA, B.T. 1985. Bivariate and multivariate growth allometry: statistical and biological considerations. J. Zool. (Lond.) 206: 367–390.

SMITH, R.J. 1984. Determination of relative size: the "criterion of subtraction" problem in allometry. J. Theor. Biol. 108: 131–142.

SMOUSE, P.E., J.C. LONG, AND R.R. SOKAL. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35: 627–632.

SOKAL, R.R. 1979. Testing statistical significance of geographic variation patterns. Syst. Zool. 28: 227–232.

SOKAL, R.R., AND N.L. ODEN. 1978. Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc. 10: 199–228.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry. W.H. Freeman and Co., New York, N.Y. 859 p.

SOMERS, K.M. 1989. Allometry, isometry and shape in principal components analysis. Syst. Zool. 38: 169–173.

SPRENGER, M.D., A.W. MCINTOSH, AND S. HOENIG. 1988. Concentrations of trace elements in yellow perch (*Perca flavescens*) from six acidic lakes. Water Air Soil. Pollut. 37: 375–388.

STRAUSS, R.E. 1985. Static allometry and variation in body form in the South American catfish genus Corydoras (*Callichthyidae*). Syst. Zool. 34: 381–396.

TAYLOR, E.B., AND J.D. MCPHAIL. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42: 2020–2028.

THORPE, R.S. 1976. Biometric analysis of geographic variation and racial affinities. Biol. Rev. 51: 407–452.

THORPE, R.S., AND L. LEAMY. 1983. Morphometric studies in inbred and hybrid house mice (*Mus* sp.): multivariate analysis of size and shape. J. Zool. (Lond.) 199: 421–432.

TRACY, C.R., AND J. SUGAR. 1989. Potential misuse of ANCOVA: comment on Packard and Boardman. Physiol. Zool. 62: 993–997.

WHITTLE, D.M., AND J.D. FITZSIMONS. 1983. The influence of the Niagara River on contaminant burdens of Lake Ontario biota. J. Great Lakes Res. 9: 295–302.

WIENER, J.G., R.E. MARTINI, T.B. SHEFFY, AND G.E. GLASS. 1990. Factors influencing mercury concentrations in walleyes in northern Wisconsin lakes. Trans. Am. Fish. Soc. 119: 862–870.

WILLIS, D.W. 1989. Proposed standard length–weight equation for northern pike. N. Am. J. Fish. Manage. 9: 203–208.

WREN, C.D., AND H.R. MACCRIMMON. 1983. Mercury levels in the sunfish, *Lepomis gibbosus*, relative to pH and other environmental variables of Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 40: 1737–1744.

WREN, C.D., W.A. SCHEIDER, D.L. WALES, B.W. MUNCASTER, AND I.M. GRAY. 1991. Relation between mercury concentrations in walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*) in Ontario lakes and influence of environmental factors. Can. J. Fish. Aquat. Sci. 48: 132–139.

# Mitochondrial DNA Variation among Lake Trout (*Salvelinus namaycush*) Strains Stocked into Lake Ontario

Peter M. Grewe[1] and Charles C. Krueger[2]

*Department of Natural Resources, Fernow Hall, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA*

Charles F. Aquadro

*Department of Genetics and Development, Cornell University, Ithaca, NY 14853, USA*

Eldredge Bermingham

*Smithsonian Tropical Research Institute, Unit 0948 APO, AA, 34002-0948, USA*

Harold L. Kincaid

*National Fishery Research and Development Laboratory, U.S. Fish and Wildlife Service, Rural Delivery 4, Box 63, Wellsboro, PA 16901, USA*

and Bernie May

*Cornell Laboratory for Ecological and Evolutionary Genetics, Department of Natural Resources, Fernow Hall, Cornell University, Ithaca, NY 14853, USA*

Grewe, P.M., C.C. Krueger, C.F. Aquadro, E. Bermingham, H.L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. 50: 2397–2403.

Mitochondrial DNA (mtDNA) variation was examined in 492 fish representing six lake trout (*Salvelinus namaycush*) strains used for stocking and restoring populations in Lake Ontario. mtDNA was extracted from 432 fish by a total DNA isolation protocol (CTAB). mtDNA was also extracted from 60 additional fish using the purification method of CsCl ultracentrifugation. The more rapid CTAB protocol made feasible analysis of sample sizes ($n \geq 80$ per strain) required as baseline data for future mixed-stock analysis (MSA). Restriction enzymes *Ava*I, *Bam*HI, *Hin*fI, and *Taq*I resolved seven mtDNA haplotypes and were used to characterize fish from each of six strains (Clearwater, Jenny, Killala, Manitou, Seneca, and Superior). Frequencies of these haplotypes were significantly different among the six strains ($p < 0.001$). Differences between haplotype frequencies of the Killala and Superior strains were striking and permit greater discrimination of these strains than allozyme data. The level of differentiation observed among strains indicates that mtDNA haplotype information will enhance the ability of MSA to determine the hatchery strains that serve as parents to lake trout fry collected from Lake Ontario.

La variation de l' ADN mitochondrial (ADNmt) a été étudiée chez 492 poissons représentant six souches de touladi (*Salvelinus namaycush*) utilisées pour l'ensemencement et le rétablissement des populations dans le lac Ontario. L'ADNmt a été extrait chez 432 poissons selon un protocole d'isolement de l'ADN total (CTAB).On a également extrait ce type d'ADNmt chez 60 autres poissons selon une méthode de purification par ultracentrifugation en CsCl. La méthode plus rapide du CTAB a permis d'analyser le nombre d'individus ($n \geq 80$ par souche) nécessaires comme données de base en vue de l'analyse future de stocks mélangés. Des enzymes de restriction *Ava*I, *Bam*HI, *Hin*fI et *Taq*I ont établi sept haplotypes d'ADNmt et ont été utilisées pour distinguer les caractères des poissons de chacune des six souches (Clearwater, Jenny, Killala, Manitou, Seneca et Superior). Les fréquences de ces haplotypes étaient significativement différentes dans les six souches ($p < 0{,}001$). Les différences entre la fréquence des haplotypes des souches Killala et Superior étaient marquées et elles ont permis de mieux distinguer ces souches que les données sur les allozymes. Le niveau de différentiation observé entre les souches montre que grâce aux données sur les haplotypes d'ADNmt, l'analyse de stocks mélangés permettra de mieux déterminer les souches provenant de piscifactures qui servent de parents aux alevins de touladi prélevés dans le lac Ontario.

*Received October 6, 1992*
*Accepted May 31, 1993*
*(JB647)*

*Reçu le 6 octobre 1992*
*Accepté le 31 mai 1993*

Lake trout (*Salvelinus namaycush*) have been stocked into Lake Ontario since 1973 as part of a program to reestablish self-perpetuating populations that disappeared from the lake in the mid-1950's due to predation by sea lamprey (*Petromyzon marinus*), habitat degradation, and overfishing. Achievement of this species restoration goal requires agencies to reintroduce strains that will survive to maturity and successfully reproduce (Schneider et al. 1983; Eshenroder et al. 1984). Analysis of fin-clip and coded wire tag data has permitted comparison of strain performance after stocking (e.g., Elrod and

[1]Present address: CSIRO Division of Fisheries, GPO Box 1538, Hobart, Tasmania, Australia 7001.

[2]Author to whom correspondence should be addressed.

Schneider 1986) but has not allowed assessment of reproductive success (i.e., fry production) in the wild. Comparison of reproductive success among lake trout strains in Lake Ontario has been identified by management agencies as a research priority (Schneider et al. 1983). Efforts to examine natural reproduction of lake trout in Lake Ontario have intensifed since 1986 when substantial numbers of fry were collected near the eastern shore of the lake (Marsden et al. 1988).

Evaluation of reproductive success requires the use of genetic markers that can discrminate among stocked strains (e.g., allozymes or mitochondrial DNA (mtDNA); Grewe and Hebert 1988; Ihssen et al. 1988; Krueger et al. 1989). Analysis of allozyme variation among strains stocked into Lake Ontario revealed the presence of 18 polymorphic loci that could be used as informative characters to genetically differentiate strains (Krueger et al. 1989). These data were subsequently used in mixed-stock analysis (MSA) to determine the parents that contributed gametes to a sample of wild fry captured in 1986 from Stony Island reef, Lake Ontario (Marsden et al. 1989). Fry in the mixture sample could have resulted from 10 potential inter- and intrastrain crosses among four strains of hatchery-origin lake trout previously stocked in Lake Ontario (Clearwater, Manitou, Seneca, and Superior strains). Since 1986, two additional strains (Jenny and Killala) have also matured. These six strains could now produce 21 potential inter- and intrastrain fry types. Unfortunately, the Killala and Superior strains exhibit a low level of differentiation from each other as detected by allozyme analysis (Krueger et al. 1989). The low level of genetic differentiation in combination with an expanded number of potential fry types substantially reduces the precision of MSA estimates of parental strain contributions (Wood et al. 1987; Marsden et al. 1989). Therefore, additional genetic characters were needed to better differentiate the parental strains and improve precision of future MSA estimates.

mtDNA restriction site polymorphisms can potentially provide an additional genetic character for use in combination with allozyme markers to identify lake trout strains. The resolution capability of mtDNA studies appears to be enhanced by the rapid evolutionary rate of mtDNA, estimated for some vertebrates to be 6–10 times the rate of the nuclear genome (Brown et al. 1979). A preliminary survey of lake trout revealed 13 mtDNA haplotypes among the Clearwater, Killala, Lewis, Manitou, Seneca, and Superior hatchery strains (Grewe and Hebert 1988). However, no mtDNA studies have been conducted with large enough sample sizes ($n \geq 80$ fish per strain) to quantitatively estimate the level of differentiation among strains and provide baseline data for MSA.

The present study describes and compares mtDNA variation among six strains of lake trout stocked into Lake Ontario. The data presented herein document the mtDNA baseline data to be used in future MSA of fry mixtures collected from Lake Ontario.

## Methods

### Sample Size Considerations

The desired precision, and hence the sample size used, for measurement of the mtDNA haplotype variation in each lake trout strain was set to provide confidence that 95% of the time, at least one individual will be detected of every haplotype present at proportions ($P$) greater than or equal to 5%. In order to determine the minimum sample size ($n$) required to satisfy this condition, we used $(1 - P)^n$, the probability of not observing a haplotype in a random sample of $n$ individuals (see Schwager et al. 1990). The probability ($\beta$) of observing at least one individual with a specified haplotype in $n$ randomly selected individuals is

$$(1) \quad \beta = 1 - (1 - P)^n .$$

Calculation of the minimum sample size required to have a certain probability or confidence ($\beta$) that at least one individual with a specifed haplotype, present at $P \geq 0.05$, will be observed in a random sample of $n$ individuals can be obtained by rearrangement of equation (1):

$$(2) \quad n = \frac{\ln(1 - \beta)}{\ln(1 - P)} .$$

Solving equation (2) by substituting the desired probability of 0.95 (95% confidence ) for $\beta$ and 0.05 for $P$ gives 58.4 individuals. Thus a minimum sample size of approximately 60 individuals is required to observe with probability 0.95 at least one individual containing a haplotype that has a proportion of $P \geq 0.05$ in a population (Fig.1). In contrast, given $n = 15$, a sample size typical of many mtDNA surveys, haplotypes present at a population frequency $P < 0.181$ will be missed 5% of the time, and 50% of the time the haplotypes present at $P < 0.045$ will remain undetected. For the present study, a more conservative minimum sample size of n = 80 was chosen. This sample size gives 98.4% confidence that at least one individual should be observed of those haplotypes present at a true population frequency of $P \geq 0.05$.

### Collections

Four hundred ninety-two fish of the Clearwater, Jenny, Killala, Manitou, Seneca, and Superior lake trout strains were analyzed. Samples of strains were obtained from the various hatcheries used to stock Lake Ontario (Table 1). These samples represented progeny from descendants of fish originally collected from various locations in the Great Lakes watershed with the exception of the Clearwater strain, which orginated from Clearwater Lake in northern Manitoba, Canada (Fig.2). Most fish sampled (432 of 492 individuals) were either fingerling or yearling trout. Effort was made to randomly sample hatchery fish that were progeny of the entire broodstock (e.g., from several raceways). Fish were frozen on site in liquid nitrogen and stored at −80°C until analyzed. The 60 remaining individuals were representative of the Clearwater, Killala, and Superior lake trout strains that were previously analyzed by Grewe and Hebert (1988).

### DNA Isolation and Restriction Analysis

Total DNA was isolated from frozen tissue samples (liver or white musle) using a modified CTAB (hexadecyltrimethylammonium bromide) isolation procedure (Saghai-Maroof et al. 1984; Doyle and Doyle 1987). Frozen tissue (10–20 mg) was homogenized in 700 µL of extraction buffer (50 mM Tris (pH 8.0), 0.7 M NaCl, 10 mM EDTA, 1% CTAB, 0.1% 2-mercaptoethanol). One hundred micrograms of proteinase K was added and samples were incubated at 60°C for 30–60 min. Samples were then extracted once with chloroform – isoamyl alcohol (24:1), once with phenol – chloroform – isoamyl alcohol

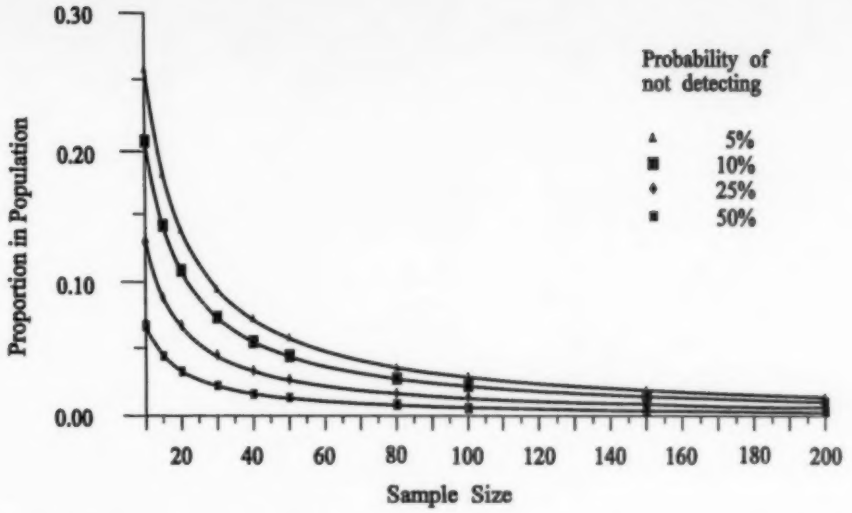



FIG. 1. Probabilities (0.05, 0.10, 0.25, and 0.50) that at least one individual of each haplotype that occurs at a proportion $P$ in a population is observed within a sample size of $n$ individuals.

TABLE 1. Strains, sample size, year class, and origin of lake trout samples examined.

| Strain | $n$ | Year class | Origin of fish and date obtained |
|---|---|---|---|
| Clearwater | 11[a] | 1983 | Clearwater Lake gamete collection, fall 1982 |
| | 71 | 1983 | Clearwater Lake gamete collection, fall 1982 |
| Jenny | 80 | 1987 | Allegheny National Fish Hatchery, March 1988 |
| Killala | 11[a] | 1983 | Hills Lake Provincial Fish Hatchery, August 1985 |
| | 69 | 1987 | Harwood Provincial Fish Hatchery, March 1989 |
| Manitou | 80 | 1987 | Harwood Provincial Fish Hatchery, March 1989 |
| Seneca | 90 | 1989 | Bath State Fish Hatchery, Bath, N.Y., December 1989 |
| Superior | 10[a] | 1975 | Jordan River National Fish Hatchery, November 1984 |
| | 8[a] | 1981 | Jordan River National Fish Hatchery, November 1984 |
| | 20[a] | 1977 | Jordan River National Fish Hatchery, November 1985 |
| | 42 | 1987 | Allegheny National Fish Hatchery, March 1988 |

[a]Samples previously analyzed by Grewe and Hebert (1988).

(25:24:1), and then a final time with chloroform – isoamyl alcohol (24:1). The aqueous phase was then collected and mixed with an equal volume of isopropanol. Samples were then placed at –20°C for a minimum of 1h after which precipitated DNA was pelleted by centrifugation in a microfuge. The DNA pellet was washed twice with 70% ethanol, dried under vacuum, and resuspended in water.

Restriction enzymes *Ava*I, *Bam*HI, *Hin*fI, and *Taq*I were chosen for this study because they had previously revealed haplotype variability among the Clearwater, Killala, Manitou, Seneca, and Superior strains (Grewe and Hebert 1988). Restriction fragments were separated in horizontal 1.2% agarose gels submerged in a Tris–borate–EDTA (TBE) buffer system (Maniatis et al. 1982). DNA was transferred to a nylon membrane filter (Zetabind™) by southern transfer (Maniatis et al. 1982). Nylon membrane filters were probed with lake trout mtDNA(50 ng used per eight 20 × 20 cm blots probed) purified by CsCl ultracentrifugation. The probe was labeled with $^{32}P$ using Amersham's Prime Time™ random priming kit. Filters were exposed to Kodak XAR5 X-ray film for 12–48 h. Fragment sizes of restriction profiles used to describe mtDNA haplotypes are listed in Table 2. Haplotypes were classified as described by Grewe and Hebert (1988) and Grewe (1991).

## Statistical Analysis

Frequencies of mtDNA haplotypes were compared statistically among samples using contingency table analysis with the $G$-statistic (Sokal and Rohlf 1981). A significance level of $p \leq 0.05$ was used to reject the null hypothesis of homogeneity

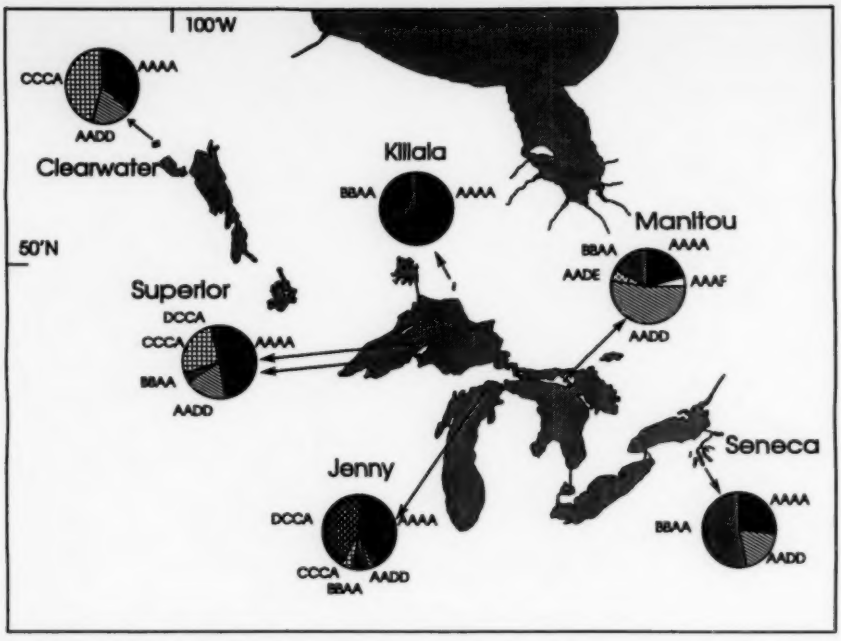



FIG. 2. Location of wild populations used to originate the Clearwater, Jenny, Killala, Manitou, Seneca, and Superior strains used for stocking into Lake Ontario. Pie diagrams display freqencies of the seven mtDNA haplotypes observed within each of the strains.

of hapalotype frequencies among strains. AAAF and AADE haplotypes, found only in the Manitou strain, were combined with the AADD haplotype category to eliminate the number of cells containing fewer than five observations in the expected contingency table.

## Results

Seven different mtDNA haplotypes were resolved among the six strains using the four restriction enzymes *Ava*I, *Bam*HI, *Hin*fI, and *Taq*I (Table 3). Six of these haplotypes (designated AAAA, AADD, AADE, BBAA, CCCA, and DCCA) were previously reported by Grewe and Hebert (1988). The AADE (observed in four individuals) and newly discovered AAAF (three individuals) haplotypes were found only among individuals of the Manitou strain. The five other common haplotypes were each present in 36 or more of the 492 fish (Table 3).

The six lake trout strains exhibited significantly different frequencies of the seven mtDNA haplotypes ($G$-value = 477.1, df = 25, $p < 0.001$). All six strains possessed the AAAA haplotype at frequencies that ranged from 21 to 67%. The Clearwater, Manitou, Seneca, and Superior strains possessed the AADD haplotype at frequecies of 18, 52, 20, and 18%, respectively. The Jenny strain also contained this haplotype but at a significantly reduced frequency (3%). The Seneca and Killala strains had the highest frequencies of the BBAA haplotype (32 and 53%, respectively) as compared with the Manitou (18%), Jenny (6%), and Superior (4%) strains. The Clearwater, Jenny, and Superior strains contained the CCCA haplotype which was not observed in the Killala, Manitou, or Seneca strains. The Jenny and Superior strains were the only ones that had the DCCA haplotype (43 and 4%, respectively). Three haplotypes (AADD, CCCA, and DCCA) found in the Superior strain at proportions of 19, 26, and 4%, respectively, were not detected in the Killala strain. Further, the BBAA haplotype occurred in the Killala strain at a frequency of 33%, but was found at a significantly lower frequency in the Superior strain (4%).

## Discussion

mtDNA haplotype variation reported here among the lake trout strains helps illustrate the caution investigators should exercise when drawing conclusions from mtDNA surveys that use small sample sizes (e.g., $n < 15$; Fig. 1). Studies that examine such small sample sizes can qualitatively demonstrate that haplotype variation exists but should not be used to characterize populations and establish MSA baseline data. For example, sample sizes ($n < 15$ for eight of the nine strains) examined by the study of Grewe and Hebert (1988) were sufficient to demonstrate the presence of most of the mtDNA haplotypes observed. This previous study, however, was insufficient to estimate haplotype frequencies at a level of precision suitable for incorporation as baseline data for MSA. In the earlier survey, a sample size of 38 was used to characterize the Superior strain but was insufficient to detect BBAA individuals. This haplotype was later observed in the present survey (4%; Table 3) when the sample size was increased to 80 fish. Data from the Manitou strain represent a more drastic example where the AAAA haplotype was present at a proportion of 21% in the final sample of 80 individuals but was missed in the earlier sample of four individuals.

Time required to analyze individuals in the laboratory (both isolation of purified mtDNA and visualization of restriction fragments) has often governed the sample sizes used to characterize populations. The total DNA isolation protocol (CTAB) used for

TABLE 2. Fragment sizes (in base pairs) for each restriction profile produced by the various enzymes. Additional fragments smaller than those reported here were present in the *Hin*fI and *Taq*I restriction digests. These fragments are not repoted here because they did not further differentiate the restriction profiles described by this study. Dashes indicate fragments missing from a specific restriction profile.

| | Restriction profile | | | | | |
|---|---|---|---|---|---|---|
| Enzyme | A | B | C | D | E | F |
| *Ava*I | 5480 | 5480 | 5480 | 5480 | | |
| | 2650 | 2650 | 2650 | 2650 | | |
| | 2210 | 2210 | — | — | | |
| | 2130 | — | — | — | | |
| | — | — | 1840 | 1840 | | |
| | 1680 | 1680 | 1680 | 1680 | | |
| | — | 1520 | 1520 | — | | |
| | 1410 | 1410 | 1410 | 1410 | | |
| | 1100 | 1100 | 1100 | 1100 | | |
| | — | — | — | 898 | | |
| | — | — | — | 622 | | |
| | — | 610 | 610 | 610 | | |
| | 565 | 565 | 565 | 565 | | |
| | — | — | 370 | 370 | | |
| *Bam*HI | 16800 | — | — | | | |
| | — | 9475 | 9475 | | | |
| | — | 7325 | — | | | |
| | — | — | 5000 | | | |
| | — | — | 2325 | | | |
| *Hin*fI | 2030 | | 2030 | 2030 | | |
| | 1260 | | 1260 | 1260 | | |
| | 1090 | | 1090 | 1090 | | |
| | 975 | | 975 | 975 | | |
| | 882 | | 882 | 882 | | |
| | — | | 827 | — | | |
| | 788 | | 788 | 788 | | |
| | 735 | | — | — | | |
| | 690 | | 690 | 690 | | |
| | — | | — | 645 | | |
| | 564 | | 564 | 564 | | |
| *Taq*I | 3670 | | | — | — | 3670 |
| | — | | | — | 2250 | — |
| | — | | | 2170 | — | — |
| | — | | | — | — | 2000 |
| | | | | 1500 | 1500 | — |
| | 1140 | | | 1140 | 1140 | 1140 |
| | 1050 | | | 1050 | 1050 | 1050 |
| | 990 | | | 990 | 990 | 990 |
| | 952 | | | 952 | 952 | 952 |
| | 933 | | | 933 | 933 | 933 |
| | 825 | | | 825 | 825 | 825 |
| | 670 | | | 670 | 670 | — |
| | 638 | | | 638 | 638 | 638 |

the present study permitted sufficient quantities of DNA (enough for 20 restriction digests from 10 mg of tissue) to be extracted from 80 individuals per day. Further decreases in sample processing time can be achieved through techniques that speed visualization time of restriction fragments. For example, amplification of small sections of the mitochondrial genome can be achieved through polymerase chain reaction (PCR) techniques to produce sufficient quantities of pure mtDNA. Restriction profiles of this DNA can then be visualized through ethidium bromide staining, rather than by the more time-consuming method of Southern blotting. Thus, given the speed of current techniques now available, the time required to process individuals should not be a factor that determines the total number of individuals sampled from populations. Reduced processing time will help increase sample sizes and will permit stronger conclusions to be drawn about population structure.

mtDNA in lake trout provides another useful genetic character that improves, over use of allozyme data only, the capability to discriminate among the six hatchery strains. An example of the advantage gained with mtDNA data is illustrated by the level of differentiation between the Killala and Superior hatchery strains which originated within close proximity to each other in the Lake

TABLE 3. Numbers and frequency of mtDNA haplotypes among six lake trout strains assayed with *Ava*I, *Bam*HI, *Hin*fI, and *Taq*I. Values in parentheses are 95% confidence limits of the frequency estimates. Four-letter haplotype designations correspond to previously described fragment patterns of *Ava*I, *Bam*HI, *Hin*fI, and *Taq*I (Grewe and Hebert 1988; Grewe 1991).

| | Haplotype designation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | AAAA | AAAF | AADD | AADE | BBAA | CCCA | DCCA | Total |
| Clearwater | | | | | | | | |
| *n* | 30 | — | 15 | — | — | 37 | — | 82 |
| Frequency | 0.366 | — | 0.183 | — | — | 0.451 | — | |
| | (0.26–0.47) | — | (0.10–0.27) | — | — | (0.34–0.56) | — | |
| Jenny | | | | | | | | |
| *n* | 35 | — | 2 | — | 5 | 4 | 34 | 80 |
| Frequency | 0.438 | — | 0.025 | — | 0.063 | 0.050 | 0.425 | |
| | (0.33–0.55) | — | (0.00–0.06) | — | (0.01–0.12) | (0.00–0.10) | (0.32–0.53) | |
| Killala | | | | | | | | |
| *n* | 54 | — | — | — | 26 | — | — | 80 |
| Frequency | 0.675 | — | — | — | 0.325 | — | — | |
| | (0.57–0.78) | — | — | — | (0.22–0.43) | — | — | |
| Manitou | | | | | | | | |
| *n* | 17 | 3 | 42 | 4 | 14 | — | — | 80 |
| Frequency | 0.213 | 0.038 | 0.525 | 0.050 | 0.175 | — | — | |
| | (0.12–0.30) | (0.00–0.08) | (0.42–0.63) | (0.00–0.10) | (0.09–0.26) | — | — | |
| Seneca | | | | | | | | |
| *n* | 24 | — | 18 | — | 48 | — | — | 90 |
| Frequency | 0.267 | — | 0.200 | — | 0.533 | — | — | |
| | (0.18–0.36) | — | (0.12–0.28) | — | (0.43–0.64) | — | — | |
| Superior | | | | | | | | |
| *n* | 38 | — | 15 | — | 3 | 21 | 3 | 80 |
| Frequency | 0.473 | — | 0.188 | — | 0.038 | 0.263 | 0.038 | |
| | (0.37–0.58) | — | (0.10–0.27) | — | (0.00–0.08) | (0.17–0.36) | (0.00–0.08) | |

Superior basin (Fig. 2). These two strains were more similar to each other allozymically than either was to the other four strains (Krueger et al. 1989). However, differences between the mtDNA haplotype frequencies of the Killala and Superior strains were striking (Table 3). The addition of mtDNA data to the allozyme baseline data used by MSA increases the detectable genetic differences among strains and thus will inprove the accuracy of MSA in determining the parental contribution to naturally produced fry in mixture samples (e.g., Pella and Milner 1987).

Construction of baseline data will be different between allozyme and mitochondrial data when MSA is to be used for second-generation analysis of fry mixtures. These differences occur because allozyme characters demonstrate typical biparental inheritance whereas mtDNA haplotypes are maternally inherited. For each strain, mtDNA data in the form of haplotype frequencies can be added to an allozyme baseline data set simply as an additional "locus" with haplotypes as "alleles." However, data must also be constructed to characterize potential interstrain crosses (e.g., Seneca × Superior). For allozyme data, the genotypic frequencies for interstrain crosses can be determined by using a modified Hardy–Weinberg equation as described by Marsden et al. (1989). Because of maternal inheritance, mtDNA baseline data used to characterize a potential interstrain cross instead should be the average of the haplotype frequencies of the two strains which assumes that the female to male ratio is the same for both strains.

In summary, the mtDNA haplotype variation observed among the Clearwater, Jenny, Killala, Manitou, Seneca, and Superior lake trout strains revealed a substantial level of differentiation among strains. These data will enhance the ability of MSA to detect parental strain contributions to lake trout fry collected from Lake Ontario.

## Acknowledgments

The following individuals provided helpful assistance in obtaining fish samples: D. Ostergaard and H. Zumstein at the Allegheny National Fish Hatchery, U.S. Fish and Wildlife Service (Jenny, Seneca, and Superior strains), and K. Wood and G. Hooper at the Harwood Fish Hatchery, Ontario Ministry of Natural Resources (Killala and Manitou strains). L. Beal assisted with mtDNA techniques, and S. Schwager advised on statistical procedures. This work is a result of research sponsored by the NOAA Office of Sea Grant, U.S. Department of Commerce, under grant No. NA86AA-D-SG045. This work was also supported by the U.S. Fish and Wildlife Service through Research Work Order No. 6 from the New York Cooperative Fish and Wildlife Research Unit and the National Fishery Research and Development Laboratory in Wellsboro, Pennsylvania. Additional support was also provided by the New York State College of Agriculture and Life Sciences, Cornell University, Hatch Projects 1476402 and 1477402.

## References

BROWN, W.M., J.R. GEORGE, AND A.C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76: 1967–1971.

DOYLE, J.J., AND J.L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytol. Bull. 19: 11–15.

ELROD, J., AND C.P. SCHNEIDER. 1986. Evaluation of coded wire tags for marking lake trout. N. Am. J. Fish. Manage. 6: 264–271.

ESHENRODER, R.L., T.P. POE, AND C.H. OLVER. 1984. Strategies for rehabilitation of lake trout in the Great Lakes: Proceedings of a conference on lake trout research, August 1983. Great Lakes Fish. Comm. Tech. Rep. No. 40.

GREWE, P.M. 1991. Temporal and spatial variability of allozyme and mitochondrial DNA markers among samples of naturally produced lake trout (*Salvelinus namaycush*) fry collected from Lake Ontario. Ph.D. thesis, Cornell University, Ithaca, N.Y. 78 p.

GREWE, P.M., AND P.D.N. HEBERT. 1988. Mitochondrial DNA diversity among broodstocks of the lake trout, *Salvelinus namaycush*. Can. J. Fish Aquat. Sci. 45: 2114–2122.

IHSSEN, P.E., J.M. CASSELMAN, G.W. MARTIN, AND R.B. PHILLIPS. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45: 1018–1029.

KRUEGER, C.C., J.E. MARSDEN, H.L. KINCAID, AND B. MAY. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. Trans. Am. Fish Soc.118: 317–330.

MANIATIS, T., E.F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MARSDEN, J.E., C.C. KRUEGER, AND B. MAY. 1989. Identification of parental origins of naturally produced lake trout fry in Lake Ontario: application of mixed-stock analysis to a second generation. N. Am. J. Fish. Manage. 9: 257–268.

MARSDEN, J.E., C.C. KRUEGER, AND C.P. SCHNEIDER.1988. Evidence of natural reproduction by stocked lake trout in Lake Ontario. J. Great Lakes Res. 14: 3–8.

PELLA, J.J., AND G.B. MILNER. 1987. Use of genetic marks in stock composition analysis, p. 247–276. *In* N. Ryman and F. Utter [ed.] Population genetics and fishery management. University of Washington Press, Seattle, Wash.

SAGHAI-MAROOF, M.A., K.M. SOLIMAN, R.A. JORGENSEN, AND R.W. ALLARD. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81: 8014–8018.

SCHNEIDER, C.P., D.P. KOLENOSKY, AND D.B. GOLDTHWAITE. 1983. A joint plan for the rehabilitation of lake trout in Lake Ontario. Lake Ontario Committee. Great Lakes Fishery Commission, Ann Arbor, Mich.

SCHWAGER, S.J., M.A. MUTCHLER, W.T. FEDERER, AND B. T. SCULLY. 1990. The effect of linkage on sample size determination for multiple trait selection. Tech. Rep. No. BU-1031-MA, Biometrics Unit, Cornell University, Ithaca, N.Y.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry. 2nd ed. W.H. Freeman and Co., San Francisco, Calif.

WOOD, C.C., S. MCKINNELL, T.J. MULLIGAN, AND D.A. FOURNIER. 1987. Stock identification with the maximum likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

# Energy Reserve Lipids of Zooplanktonic Crustaceans from an Oligotrophic Saline Lake in Relation to Food Resources and Temperature

Michael T. Arts, Richard D. Robarts, and Marlene S. Evans

*National Hydrology Research Institute, Environmental Sciences Division, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada*

Arts, M.T., R.D. Robarts, and M.S. Evans. 1993. Energy reserve lipids of zooplanktonic crustaceans from an oligotrophic saline lake in relation to food resources and temperature. Can. J. Fish. Aquat. Sci. 50: 2404–2420.

In three zooplankton species examined over a 2-yr period in an oligotrophic saline (22 g · $L^{-1}$) lake, triacylglycerols (energy reserve lipids) were the most abundant lipid class followed by phospholipids and sterols. Marked seasonal differences in patterns of total and energy reserve lipid content in the herbivorous calanoid *Leptodiaptomus sicilis* were correlated with temporal patterns in edible algal biomass and temperature. The appearance of particular algal species, or groups of similar-sized species, occurred synchronously with changes in lipid content of both *L. sicilis* and *Daphnia pulex*. These periods of changing lipid content were used to infer nutritional suitability or inadequacy (unavailability) of specific algae for wild populations of zooplankton. Energy transfer from phytoplankton to the carnivorous calanoid *Hesperodiaptomus nevadensis* through *L. sicilis* involved a time lag of approximately 1–2 mo; this was hypothesized to result from a feeding dependency on *L. sicilis* copepodites due to a gape limitation of the predator. Demographics of *D. pulex* in this lake were unusual because the species was consistently absent during spring. In addition, total lipids and triacylglycerols were low (<15% and <4.5 μg · $animal^{-1}$, respectively), suggesting that *D. pulex* had been pushed into a subsistence niche by the rising salinity levels.

L'examen pendant une période de deux ans de trois espèces de zooplancton dans un lac salin oligotrophe (22 g · $L^{-1}$) a montré que les triacyl-glycérols (lipides de réserve) étaient la classe de lipides la plus abondante suivie des phospholipides et des stérols. Des variations saisonnières prononcées des profils de la teneur en lipide total et en lipides de réserve chez *Leptodiaptomus sicilis*, calanoïde herbivore, ont été corrélées à des profils temporels de la biomass algale comestible et à la température. La présence d'une espèce d'algue particulière, ou groupes d'espèces de même taille, était synchronisée avec des changements de la teneur en lipides chez *L. sicilis* et *Daphnia pulex*. Ces périodes de variation de la teneur en lipides ont été utilisées pour mesurer la disponibilité ou la valeur de certaines algues pour l'alimentation des populations sauvages de zooplancton. Le transfert d'énergie du phytoplancton au calanoïde herbivore *Hesperodiaptomus nevadensis* par *L. sicilis* comprenait un délai d'environ un à deux mois; on a supposé que cette situation était attribuable à la dépendance alimentaire aux copépodes *L. sicilis* en raison de la limite du prédateur. Les donnéees démographiques de *D. pulex* dans ce lac étaient surprenantes parce que l'espèce était régulièrement absente au printemps. De plus, les lipides totaux et les triacyl-glycérols étaient faibles (respectivement < 15 % et < 4,5 μg · $animal^{-1}$) ce que semble indiquer que l'augmentation du taux de salinité poussait *D. pulex* à se trouver une niche lui permettant de subsister.

*Received November 3, 1992*
*Accepted May 21, 1993*
*(JB681)*

*Reçu le 3 novembre 1992*
*Accepté le 21 mai 1993*

Lipids, in particular neutral lipids such as triacylglycerols and wax esters, are important reservoirs and conveyors of energy through marine and freshwater invertebrate food webs. Many studies document seasonal patterns in the standing stock of these energy-storing molecules in marine systems (Sargent and Henderson 1986) and increasingly in freshwater systems (Arts et al. 1992; Vanderploeg et al. 1992). Very few studies, however, have examined lipid dynamics in athalassic saline lakes (but see Hairston 1979). The paucity of studies undertaken on seasonal dynamics of energy reserve lipids in plankton of athalassic saline lakes is conspicuous given that the total volume of the world's saline lakes is only slightly less (83%) than that of freshwater lakes (Hammer 1986). Saline lakes are common in areas with endorheic drainage basins where precipitation is exceeded by evaporation. Saline lakes of midwestern North America, and Saskatchewan in particular, have ion proportions that are often very different from those of the ocean (Hammer 1978). Sodium, magnesium, and sulphate ions tend to dominate, although the exact cation and anion compositions in saline lakes are often variable (Hammer 1978).

Decreases in species richness along gradients of increasing salinity, as a result of salt intolerance, have been reported in prairie saline lakes for various trophic levels: fish (Rawson and Moore 1944), zooplankton (Moore 1952), benthos (Timms et al. 1986; Hammer et al. 1990), phytoplankton (Hammer et al. 1983), and macrophytes (Hammer and Heseltine 1988). It may be potentially easier to demonstrate whether or not seasonal and species-specific changes in lipid energy reserves of herbivorous zooplankton are correlated with temporal changes in the standing stocks of different species of algae in saline lakes, as compared with freshwater lakes, because food web complexity (sensu Sprules and Bowerman 1988) is reduced by high-salinity levels.

These alterations in community structure result in simplified and truncated food webs when, for example, large piscivores and planktivores are reduced or eliminated. The present-day salinity of Redberry Lake, in central Saskatchewan, exceeds tolerances of most fish species indigenous to the area (see Rawson and Moore 1944). Presently, brook stickleback (*Culaea inconstans*) are common in inshore regions. Walleye (*Stizostedion vitreum*), rainbow trout (*Oncorhynchus mykiss*), and especially lake whitefish (*Coregonus clupeaformis*) have been stocked at various times over a 46-yr period (1940–86). Due to only "fair" survivorship and slow growth of lake whitefish and poor survival of rainbow trout, further stocking of lake whitefish and rainbow trout was terminated in 1986–87. The lack of success of these fisheries has been blamed on increasing salinity levels and supercooled under-ice water temperatures.

One of the goals of laboratory feeding studies involving zooplankton grazers is to measure and compare the encounter, rejection, ingestion, and assimilation rates of different species of algae for a given zooplankton species. Measurements of these feeding parameters and the biochemical composition of the algae could then be combined to assess the nutritional suitability of a given algal species. However, in practice, since little is known about how the biochemical parameters relate to the intrinsic nutritional qualities of algal cells, some other measure of fitness is usually substituted for the biochemical parameters in order to assess nutritional suitability. Familiar examples include growth rate, reproductive output, and lipid content. Fatty acids of dietary origin have been shown, in the laboratory, to be rapidly deposited within neutral lipid stores (wax esters and triacylglycerols), making total lipid content and, in particular, energy reserve lipid content good predictors of recent feeding history (Bourdier and Amblard 1989; Goulden and Place 1990) and potentially good indicators of a zooplankter's nutritional status (Tessier et al. 1983; but see Sterner et al. 1992 for a cautionary commentary).

A laboratory technique using this approach would have the advantage of being well controlled and replicable. Problems could arise in extrapolating the laboratory-derived results to field conditions, as the biochemical composition of the algal species of interest might be different in the laboratory compared within the field. This is because laboratory cultures of algae are usually maintained on standard media whereas in the field, nutrients and other environmental parameters that are known to affect phytoplankton biochemical composition change seasonally. It is well known, for example, that algae can alter the relative allocation of macromolecules amongst protein, carbohydrate, and lipid pools in response to changes in light intensity (Cuhel et al. 1984) or nutrients (Shifrin and Chisholm 1981). Another disadvantage is that time limitations related to measuring the fitness parameters and our inability to successfully culture many algal species precludes testing of more that a few species at a time. Finally, although multispecies assemblages of algae are common in lakes, it would be very difficult to assess the combined nutritional quality of several species of algae simultaneously in the laboratory.

It might, however, be possible, under some conditions, to complement the experimental laboratory approach with field data in order to obtain additional and unique measures of algal nutritional suitability. The conditions under which correlations between algal species composition and lipid content of herbivorous zooplankton are optimized to provide inferences concerning algal nutritional suitability include (a) periods where only one, or at most a few, edible algal species predominate, (b) short intervals during which lipid levels are changing rapidly, and (c) periods of increasing temperature accompanied by increasing lipid levels.

The advantage of the field approach is that it allows evaluation of algal nutritional suitability under real field conditions where nutrient regimes and biochemical composition of the algae are in constant flux seasonally and where normal vertical migration patterns of the zooplankton are preserved. In addition, the field approach provides a nutritional assessment of algal species that may be difficult to culture in the laboratory as well as providing some insight into the nutritional value of small species assemblages (i.e., less than two to four species). Finally, simultaneous measurement of edible algal biomass and temperature allows for the development of multivariate models with the potential to predict when lipid levels of herbivores are likely to be high or low. This is important because it leads to prediction of the conditions necessary to promote lipid energy accumulation and subsequent energy transfers within and amongst trophic levels (e.g, from prey to predator). The main disadvantages of the field approach include (a) the possibility that our incomplete knowledge of algal morphology, biochemical suitability, and digestion resistance and the effects that these variable have on ingestion rate could lead to an incorrect categorization of a particular algal species as edible or inedible and (b) the possibility that confounding factors such as parasitism and toxic stress may at times influence lipid levels independently of algal quality and quantity.

In this paper, seasonal patterns of total lipid and lipid energy reserves are compared amongst different zooplankton species comprising two herbivores and one facultative carnivore over a 2-yr period in a prairie saline lake. The two study years differed markedly with respect to phytoplankton species composition. We examine, in detail, specific transition periods in zooplankton lipid energy reserve content in relation to phytoplankton species composition and biomass. This technique has been used to infer nutritional suitability and/or inadequacy of particular species of phytoplankton for zooplankton grazers over a 1-yr period in a nearby hypereutrophic lake (Arts et al. 1992). We also examine whether seasonal rates of primary (phytoplankton) productivity, chlorophyll concentration, and secondary (bacterial) productivity are correlated with lipid levels of herbivorous zooplankton. This was done to see if these measures could be used instead of other descriptors of food quality/quantity, such as edible algal biomass or detailed taxonomic descriptions of phytoplankton assemblages, to predict seasonal changes in total lipid content or energy reserve content of zooplankton. For one of the herbivores, a copepod, we construct an empirical model which illustrates the relative importance of food and temperature as determinants of zooplankton lipid energy reserve levels.

Crucial to successful interpretation of top-down or bottom-up transfers of energy in natural systems, or during a biomanipulation, is the recognition that time lags must be incorporated into dynamic models of energy flow (Carpenter and Kitchell 1992). We trace the effects of temporal changes in the apparent quality/quantity of the edible phytoplankton fraction through a herbivorous copepod to its main predator, a carnivorous copepod, and suggest that time lags in bottom-up energy transfers can be introduced by mechanical limitations imposed by the mouthpart morphology of the predator.

## Methods

### Field Site

Redberry Lake ($Z_{max}$ = 16 m, surface area = 45 km$^2$) is situated (52°43′N, 107°09′W) on a glacial lacustrine alluvial plain underlain by Upper Cretaceous shales and sands (Hammer and Haynes 1978). The lake has been the focus of several studies over the past five decades (e.g., Rawson and Moore 1944; Haynes and Hammer 1978; Hammer et al. 1983; Timms et al. 1986; Robarts et al. 1992). The lake is saline (22 g · L$^{-1}$) with magnesium (68.1%) and sodium (29.2%) cations and sulphate (93.7%) anions dominating, oligotrophic (mean total phosphorus during spring overturn = 46.5 μg · L$^{-1}$ and maximum euphotic zone chlorophyll 5.5 μg · L$^{-1}$), and alkaline (mean pH = 8.9).

Ice formation typically begins in early November and breakup usually occurs at the beginning of May. The ice can attain a thickness of up to 1.0 m by January and below-ice temperatures can reach –0.5°C. The water column remains aerobic throughout the year, and light usually penetrates to the bottom sediments (Robarts et al. 1992).

### Physical–Chemical Parameters

Temperature was measured on a Cole-Parmer thermistor (model 8502-20) at each depth. For statistical analyses, we used a working definition of epi- (0–8 m) and hypolimnion (8–16 m) and calculated a mean epi- and hypolimnetic temperature for each date. More detailed temperature profiles can be found in Robarts et al. (1992).

Total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), particulate nitrogen (PN), ammonia ($NH_4^+$), nitrite/nitrate ($NO_2$+$NO_3$-N), silica ($SiO_2$), and dissolved and particulate organic carbon (DOC and POC) were measured from near-surface (0–0.5 m), middle (10 m), and near-bottom (16 m) waters as in Robarts et al. (1992). $NO_2$+-$NO_3$-N and SRP were also measured at 4 and 14 m (Robarts et al. 1992). Oxygen was measured at each depth by the Winkler method. Total alkalinity was determined to an end-point of pH 4.5 by titration with 0.1 M HC1, and pH was measured with a Cole-Parmer portable meter. For the statistical analyses, only the mean values for the above variables for the entire water column on any given date were used.

### Bacteria and Phytoplankton

Bacteria and phytoplankton were collected with an 8-L Niskin bottle at 2-m intervals from surface to bottom during the ice-free months and from just under the ice, at 8 m, and at 16 m during winter. Samples were collected synchronously with zooplankton collections. Bacteria and phytoplankton for standing biomass estimates were preserved with Lugol's solution.

Bacteria from each depth were concentrated on a 0.2-μm, 25-mm polycarbonate membrane filter, stained with 4′,6-diamidino-2-phenylindole (DAPI), and counted under epifluorescent light (Robarts and Sephton 1981). Bacterial numbers are reported as the mean for the epi- and hypolimnion.

Bacterial productivity was estimated as the rate of [$^3$H-methyl]thymidine incorporation into DNA from in situ measurements made at 1-m depth intervals following the method of Robarts and Wicks (1989). Water samples were collected from each depth and incubated with a saturating concentration of 18 nM TdR at the collection depth by suspending the tubes from a vertical incubation harness. Incubations proceeded for 15 min in summer and 30–60 min in winter. Preservation of the samples and prevention of further TdR incorporation was achieved by adding 0.5 mL of 5M NaOH at the end of the incubation period. DNA was then extracted and purified. Bacterial production is given as the planimetrically integrated rate of DNA labeling for the water column.

Water for chlorophyll *a* analyses (1–2 L) was drained from Niskin bottles and drawn through GF/C filters. The filters were kept on ice in the dark. Chlorophyll *a* was extracted from the filters by boiling in ethanol and measuring the fluorescence on a Turner fluorometer (model 10-005R) after correction for phaeopigments (Nusch 1980). Chlorophyll is given as the mean for the epi- and hypolimnion.

Integrated phytoplankton samples were obtained by combining 100-mL aliquots from the discrete depth intervals within the epi- and hypolimnion. A combined minimum of 500 cells, colonies, and filaments were counted per integrated sample as in Earle et al. (1987). Algae were usually identified to species level. Counts were expressed as wet biomass (milligrams per cubic meter) using simple geometric models based on linear dimensions of cells.

Edible algal cells are often represented by species belonging to the Cryptophyta, Chlorophyta, or Chrysophyta and are usually less than 40 μm in the longest dimension (Porter 1973; Ross and Nunawar 1981; Chow-Fraser 1985). Algal species were classed as potentially edible or inedible based on cell or colony size and whether or not colonies or cells were encapsulated with a gelatinous sheath. Cyanobacteria were considered inedible. Pyrrhophyta, composed of heavily armored species with thick exoskeleton, such as *Peridinium* sp. and *Gymnodinium* sp., and large ciliate species, such as *Strombilidium* sp. and *Strombidium* sp., were all considered inedible. We consider diatoms separately from other potentially edible species because the large net species, such as *Stephanodiscus niagarae* Ehr. (53.8 × 31.6 μm), *Surillela ovata* Kuetz (55.7 × 39.2 μm), and *Chaetoceras elmorei* Boyer (137.2 × 43.5 μm), which usually dominated the diatom biomass in Redberry Lake, are not normally considered a food source for *Daphnia* sp. which lack the silicacous mouthparts necessary to penetrate diatom frustules and because the mechanisms and rules governing utilization of diatoms by calanoids are complex (Vanderploeg 1988, 1990). Another complication is that large net diatoms are rarely consumed whole by copepods; rather, small pieces are commonly bitten off and the rest may remain uneaten (Vanderploeg 1988).

Primary productivity was measured using the $^{14}$C light and dark bottle technique as in Robarts et al. (1992). Primary production was calculated on the basis of $^{14}$C-incorporation rates and $^{12}$C available to phytoplankton using the conversion table of Saunders et al. (1962) and ambient temperature, pH, and alkalinity measurements. As a rough approximation of primary production attributable to edible phytoplankton, we multiplied hourly rates of primary production by the ratio of edible to inedible biomass on each sampling date. These estimates were then used in the stepwise regression analyses. We realize that these estimates do not take into account that particular species may have different growth rates or that the growth rates of smaller algae are generally proportionally greater than those of larger species (Reynolds 1984).

### Zooplankton Sampling and Lipid Analysis

Zooplankton were collected at roughly 2-wk intervals from mid-May to mid-October 1989 and at 1-mo intervals from

January 1990 to January 1991, but not during periods of thin ice. A Wisconsin-style plankton net (76-μm Nitex mesh, 0.5-m mouth diameter, 2-m length) was towed from a position 1 m off the bottom to the surface at the deepest point in the lake. The dominant zooplankton species in Redberry Lake over the study period included *Leptodiaptomus sicilis* S. A. Forbes, *Daphnia pulex* Leydig (both predominantly herbivorous species), and *Hesperodiaptomus nevadensis* (Light) (a carnivore). The omnivore *Diacyclops bicuspidatus thomasi* S. A. Forbes is also found in low abundance. Live zooplankton for lipid analyses were kept in 25-L polyethylene carboys filled with lake water. Upon returning to the laboratory, zooplankton were transferred to 25-L glass aquarium in a cold-room maintained at 4 and 8°C during the ice-covered and ice-free months, respectively. The next day, zooplankton were killed with a dilute (≈0.1%) formaldehyde solution and quickly sorted by species, sex, and reproductive condition. Because our primary interest was in determining broad, species-specific, seasonal patterns in energy reserves in relation to food resources, total lipid concentrations and lipid classes were pooled for the different sexes and reproductive conditions and only mean lipid values are reported.

Animals were placed in small test tubes (6 mm diameter × 50 mm long) held in an ice-cooled metal tray. Individual *D. pulex* were classed as adult on the basis of size. The body size of the smallest individual carrying eggs on a given date was used to estimate the minimum size at maturity. A mean of 65, 25, and 10 individuals of *L. sicilis*, *D. pulex*, and *H. nevadensis*, respectively, were used to obtain sufficient tissue mass for a singe replicate. On average, we obtained 12, 6, and 10 replicates of *L. sicilis*, *D. pulex*, and *H. nevadensis*, respectively, on each date. The test tubes were placed in a sealed desiccator (50°C) under a steady flow of nitrogen gas and allowed to dry overnight. The tubes were then placed in preserving jars, purged with nitrogen, and stored at −40°C to minimize lipid oxidation.

Total lipids were extracted from dried zooplankton tissue samples following a modification (Gardner et al. 1985) of the gravimetric technique originally developed by Folch et al. (1957). A small aliquot of this lipid extract was saved for determination of lipid class composition by thin-layer chromatography coupled with flame ionization detection (TLC-FID) after the method of Parrish (1987) on an Iatroscan MK-IV detector (Iatron Labs., Tokyo) equipped with SIII-chromarods (Ancal Inc.). The various lipid classes were quantified by integrating peak areas with a Shimadzu chromatopac (model C-R6A). Peak areas were converted to lipid concentrations using nonlinear regressions derived for each of the lipid classes. Identification of peaks in each sample was based on relative peak retentions obtained from one of the chromarods reserved for a mixed lipid standard. The mixed lipid standard consisted of equal concentrations (1 $\mu g \cdot mL^{-1}$) of the following lipid classes: hydrocarbon (HC), wax ester (WE), sterol ester (SE), triacylglycerol (TG), free fatty acid (FFA), fatty alcohol (ALC), sterol (ST), acetone-mobile polar lipid (AMPL), and phospholipid (PL). The trivial names of the standards purchased from Sigma Chemical Co. are HC = squalane, WE = stearyl palmitate, SE = 3-hexadecanone, TG = tripalmitin, FFA = palmitic acid, ALC = cetyl alcohol, ST = cholesterol, AMPL = α-monopalmitin, and PL = lecithin.

### Statistical Analyses

Stepwise multiple regression was used to identify relationships between total lipid concentration or triacylglycerol content for *L. sicilis* and selected physical and phytoplankton-related parameters for the ice-free season. The variables used in the regression analyses did not extend to the under-ice period because zooplankton lipid reserves do not appear to be tightly coupled to food resources during winter in Redberry Lake. This is based on the observation that lipid reserves are usually very high in January and February, although edible algal biomass can be very low (see also Arts et al. 1992). The negative correlation between food resources and under-ice lipid levels can be explained by the very low under-ice temperatures in Redberry Lake. Subzero temperatures depress metabolic rates of zooplankton, and any lipid deposited in the fall would decline very slowly over the subsequent winter.

A generalized three-dimensional predictive model relating mean epi-and hypolimnetic temperature and epilimnetic edible algal biomass to total lipid concentration (percentage of dry weight) was developed from the equation generated by the stepwise regression procedure. The model was constrained as follows: mean epilimnetic temperature was always greater than hypolimnetic temperature, mean epi- and hypolimnetic temperatures were between 5 and 21°C and 1 and 10°C, respectively, and edible algal biomass was either 100 or 600 $mg \cdot m^{-3}$.

## Results

### General Seasonal Patterns

Redberry Lake is thermally stratified during the ice-free season with mean epi- and hypolimnetic water temperatures ranging from 5 to 21° and 1 to 10°C, respectively (Fig. 1). Mean hypolimnetic temperatures were higher in August and September of 1990 than during the same period in 1989.

Seasonal patterns in chlorophyll *a* concentrations differed between the two years and between the epi- and hypolimnion (Fig. 2A). Overall, chlorophyll *a* concentrations were highest (>2.0 $\mu g \cdot L^{-1}$) in spring and fall of 1989 and in spring to midsummer of 1990. Chlorophyll concentrations were 2–3 times higher in the hypolimnion compared with the epilimnion in early summer of 1990 and the spring of 1989 and somewhat higher in the hypolimnion in the fall of 1989 (Fig. 2A).

Edible algal biomass exceeded 200 $mg \cdot m^{-3}$ in spring, July, and the fall of 1989 and all summer until October of 1990 (Fig. 2B). The edible algal biomass in the fall of 1990 (October) was lower than in 1989 (October). The most striking contrast in edible algal biomass between the epi- and hypolimnetic regions occurred in May through August of 1990 when hypolimnetic biomass was 1.5–2 times higher than epilimnetic biomass (Fig. 2B). Edible biomass was roughly 200 $mg \cdot m^{-3}$ during fall of 1990 when temperatures were decreasing rapidly.

Primary productivity (means for each depth horizon) reached 14 $mg\ C \cdot m^{-3} \cdot h^{-1}$ in the epilimnion of June 28 and 12 $mg\ C \cdot m^{-3} \cdot h^{-1}$ on August 10, 1989, during a bloom of *Aphanizomenon flos-aquae* (Fig. 1C). Mean primary productivity rates (Fig. 1C) remained at roughly 5 $mg\ C \cdot m^{-3} \cdot h^{-1}$ for September and October 1989. Rates of primary productivity were higher during the fall than at any other time in 1990 (Fig. 1C).

In May 1989, edible algae were dominated by small chrysophytes, notably *Chrysidalis peritaphrena*, *Cercobodo varians* and *Chromulina nitens* (Table 1). In the fall of 1989, the epilimnion was dominated by small (1–10 μm) to intermediate (10–20 μm) sized algal cells (Fig. 3C and 3D). Edible chlorophyte species(s) were especially abundant in the epilimnion

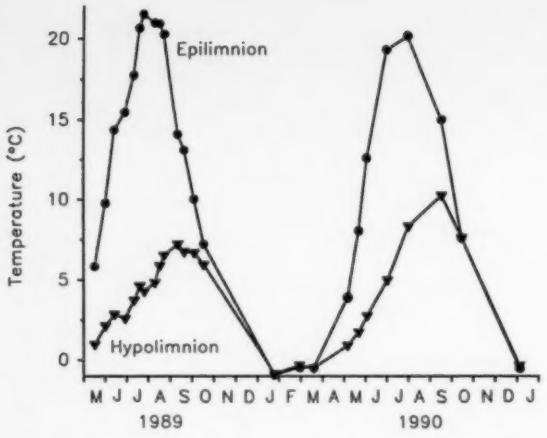



FIG. 1. Temporal patterns in mean temperature in the epilimnion (0–8 m) and hypolimnion (8–16 m) of Redberry Lake from May 1989 to January 1991.

(Fig. 3A). Chrysophyte biomass (≈1 mg · m$^{-3}$) was modest and similar for both depth strata at this time. Although cryptophyte biomass was relatively low (<1 mg · m$^{-3}$), cryptophytes occurred predominantly within deeper, cooler water in the fall of 1989 (Table 1). *Oocystis borgei*, *C. peritaphrena*, *Erkenia subaequiciliata*, and *Rhodomonas minuta* dominated the edible algal biomass in the fall of 1989 (Table 1). In March 1990, before thermal stratification was pronounced, *Euglena variablis* dominated the edible algal biomass. During the spring to midsummer period of 1990, small (1–10 μm) to intermediate (10–20 μm) sized chlorophytes (e.g., *Coccomyxa* sp.) and chrysophytes (e.g. *C. peritaphrena*, *C. varians*, *E. subaequiciliata*), as well as other chrysophytes belonging to the suborder *Chromulinineae*, dominated the edible algal biomass (Fig. 3; Table 1).

## Diatoms, Presumed Inedible Algal Species, and Bacteria

During summer of 1989 the chlorophyte, chrysophyte, and cryptophyte portion of the edible biomass made up only a small fraction of total algal biomass (Table 1). Diatoms were important contributors to algal biomass in June and July of 1989. For example, the large diatom *S. niagarae* (743.7 mg · m$^{-3}$) comprised 65.5% of the total algal standing crop in the epilimnion on June 14. Diatoms also comprised a large portion of the total biomass in the fall of 1989 and during June and July of 1990 (Fig. 4). The dominant species in the hypolimnion on September 20, 1989, were pennate diatoms (1311.9 mg · m$^{-3}$), *S. ovata* (403.4 mg · m$^{-3}$), *S. niagarae* (323.3 mg · m$^{-3}$), *Nitzschia* sp. (120.2 mg · m$^{-3}$), and *C. elmorei* (110.3 mg · m$^{-3}$). *Chaetoceras elmorei* (923.3 mg · m$^{-3}$) was the dominant diatom in the epilimnion biomass on June 4, 1990, and shortly thereafter was also prevalent in the hypolimnion (1187.7 mg · m$^{-3}$).

In May 1989, presumed inedible species included *Planktosphaeria gelatinosa* (473.1 mg · m$^{-3}$) and *Chrysocapsa planctonica* (462.2 mg · m$^{-3}$), which together comprised 55% of the total biomass from the hypolimnion on May 17.

The ciliates *Strombidium* sp. and *Strobilidium* sp. accounted for 73.9% of the total biomass (433.8 mg · m$^{-3}$) in the hypolimnion on June 14, 1989. In August 1989 the cyanobacteria *A. flos-aquae* and *Lyngbya birgei* and the chlorophyte *Gleocystis major* dominated the inedible biomass.

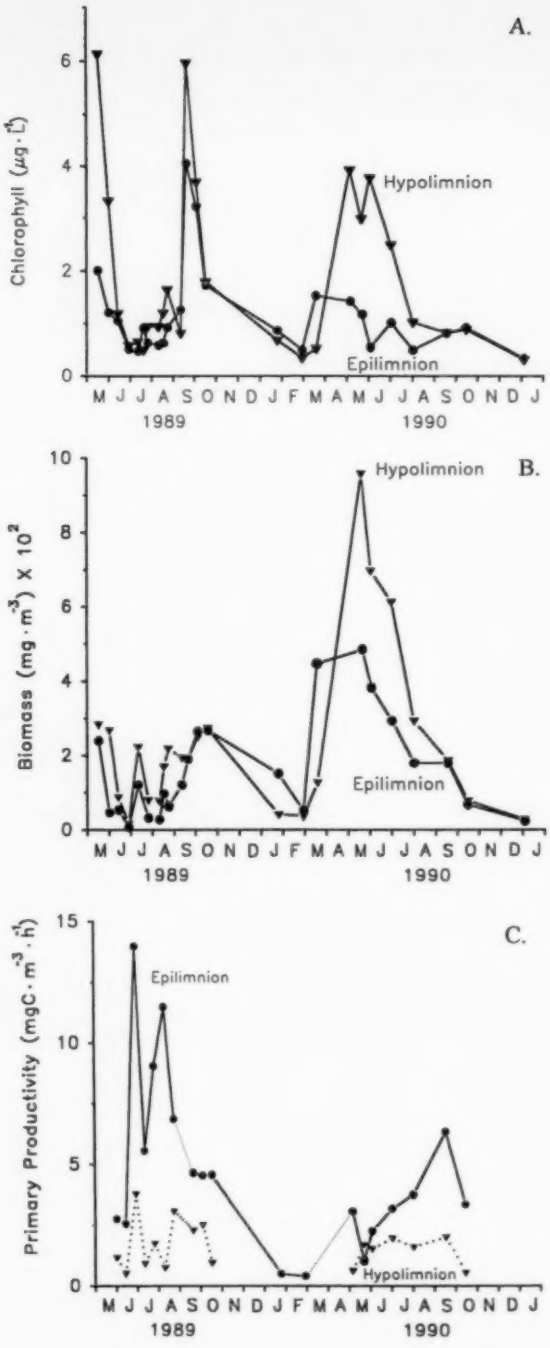



FIG. 2. Temporal patterns in (A) chlorophyll *a* concentration, (B) presumed edible algal biomass and, (C) primary productivity in the epilimnion (0–8 m) and hypolimnion (8–16 m) of Redberry Lake from May 1989 to January 1991.

Bacterial numbers were lowest just before ice-off, highest during summer, and declined sharply in the fall (Fig 5A). There was no consistent difference between the epi- and hypolimnion with respect to bacterial numbers. Bacterial productivity tended to be highest in summer and lowest over the winter months

TABLE 1. Presumed edible algal species (see text) that individually accounted for at least 5% of the total edible algal biomass on a given date. The first number below the date refers to the total biomass ($mg \cdot m^{-3}$) of all presumed edible algae for each depth strata and the second number, in parentheses to total algal biomass ($mg \cdot m^{-3}$). The column labeled "% edible biomass" refers to the proportion of total edible biomass represented by each species for that depth.

| Date | Depth | Taxa | Species | Biomass ($mg \cdot m^{-3}$) | % edible biomass | Length (μm) | Width (μm) |
|---|---|---|---|---|---|---|---|
| May 17, 1989 | Epilimnion | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 198.6 | 82.9 | 5.1 | 5.1 |
| 239.7 (581.5) | | | *Ochromonas globosa* Skuja | 23.2 | 9.7 | 7.6 | 7.6 |
| | Hypolimnion | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 105.8 | 37.3 | 5.1 | 5.1 |
| 284.0 (1686.6) | | | *Chromulina nitens* Skuja | 61.3 | 21.6 | 14.2 | 10.7 |
| | | | Chrysophyta unidentified | 45.6 | 16.1 | 18.0 | 14.2 |
| | | | Chrysophyta unidentified | 45.2 | 15.9 | 14.2 | 13.0 |
| | | | *Pleuromonas nastua* Skuja | 16.3 | 5.8 | 8.3 | 5.9 |
| June 1, 1989 | Epilimnion | Chrysophyta | *Cercobodo varians* Skuja | 20.0 | 42.9 | 11.9 | 8.0 |
| 46.7 (119.3) | | | *Sennia parvula* Skuja | 6.9 | 14.8 | 5.2 | 4.2 |
| | | | Chrysophyta unidentified | 6.0 | 12.9 | 10.8 | 7.2 |
| | | | *Chrysidalis peritaphrena* Schill. | 3.2 | 6.9 | 5.1 | 5.1 |
| | | | *Pleuromonas nastua* Skuja | 3.1 | 6.7 | 8.3 | 5.9 |
| | Hypolimnion | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 75.0 | 28.0 | 5.1 | 5.1 |
| 268.1 (717.2) | | | *Cercobodo varians* Skuja | 70.2 | 26.2 | 11.9 | 8.0 |
| | | | *Erkenia subaequiciliata* Skuja | 58.6 | 21.8 | 4.7 | 4.0 |
| | | | *Pleuromonas nastua* Skuja | 35.1 | 13.1 | 8.3 | 5.9 |
| | | | *Ochromonas lenta* Skuja | 23.8 | 8.9 | 16.1 | 8.4 |
| June 14, 1989 | Epilimnion | Chrysophyta | Chrysophyta unidentified | 18.0 | 33.5 | 10.6 | 7.0 |
| 53.5 (1135.8) | | | *Chrysidalis peritaphrena* Schill. | 16.7 | 31.2 | 5.1 | 5.1 |
| | | | *Pleuromonas nastua* Skuja | 2.9 | 5.5 | 8.3 | 5.9 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 7.9 | 14.8 | 10.7 | 5.5 |
| | Hypolimnion | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 52.1 | 59.8 | 5.1 | 5.1 |
| 87.2 (587.2) | | | *Cercobodo varians* Skuja | 12.8 | 14.6 | 11.9 | 8.0 |
| | | | *Pleuromonas nastua* Skuja | 8.2 | 9.4 | 8.3 | 5.9 |
| | | | *Sennia parvula* Skuja | 7.6 | 8.7 | 5.2 | 4.2 |
| June 28, 1989 | Epilimnion | Chlorophyta | *Chlamydomonas gloeophila* Skuja | 0.6 | 8.3 | 10.7 | 3.6 |
| 6.7 (105.8) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 5.2 | 77.1 | 5.1 | 5.1 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 0.4 | 6.5 | 10.7 | 5.5 |
| | Hypolimnion | Chrysophyta | *Cercobodo varians* Skuja | 5.8 | 35.9 | 11.9 | 8.0 |
| 16.2 (121.6) | | | *Sennia parvula* Skuja | 2.7 | 16.3 | 5.2 | 4.2 |
| | | | *Chrysidalis peritaphrena* Schill. | 2.0 | 12.5 | 5.1 | 5.1 |
| | | | *Pteridomonas pulex* Penard. | 2.0 | 12.5 | 7.8 | 7.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 1.0 | 5.9 | 10.7 | 5.5 |
| July 12, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 9.2 | 7.7 | 18.5 | 12.6 |
| 119.9 (509.0) | | Chrysophyta | *Monochrysis aphanaster* Skuja | 9.4 | 7.8 | 7.6 | 6.6 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 68.6 | 57.2 | 10.7 | 5.5 |
| | | | *Katablepharis ovalis* Skuja | 8.9 | 7.4 | 7.3 | 4.3 |
| | | | *Rhodomonas minuta* v. *nannoplanctica* Skuja | 8.0 | 6.7 | 8.4 | 4.5 |
| | Hypolimnion | Chrysophyta | *Cercobodo varians* Skuja | 183.9 | 82.8 | 11.9 | 8.0 |
| 222.2 (428.3) | | | *Chrysidalis peritaphrena* Schill. | 21.9 | 9.8 | 5.1 | 5.1 |
| July 26, 1989 | Epilimnion | Chlorophyta | *Oocystis parva* West & West | 17.6 | 54.4 | 11.0 | 6.1 |
| 32.3 (941.5) | | | *Chrysapsis sagene* Pasch. | 2.0 | 6.3 | 3.3 | 3.3 |
| | | | *Cyanomonas americana* (Davis) Oltmanns | 3.2 | 9.8 | 5.7 | 3.4 |
| | | | *Rhodomonas minuta* Skuja | 3.1 | 9.4 | 10.7 | 5.5 |
| | | | *Rhodomonas minuta* v. *nannoplanctica* Skuja | 2.2 | 6.8 | 8.4 | 4.5 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 29.7 | 37.3 | 18.5 | 12.6 |
| 79.6 (686.8) | | | *Oocystis parva* West & West | 7.0 | 8.7 | 10.7 | 6.1 |
| | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 16.1 | 20.2 | 5.1 | 5.1 |
| | | | *Sennia parvula* Skuja | 6.9 | 8.7 | 5.2 | 4.2 |

TABLE 1. (*Continued*)

| Date | Depth | Taxa | Species | Biomass ($mg \cdot m^{-3}$) | % edible biomass | Length (μm) | Width (μm) |
|---|---|---|---|---|---|---|---|
| Aug. 10, 1989 | Epilimnion | Chlorophyta | *Oocystis parva* West & West | 3.7 | 13.4 | 10.7 | 6.1 |
| 27.8 (483.8) | | Chrysophyta | *Sennia parvula* Skuja | 9.1 | 32.7 | 5.2 | 4.2 |
| | | | Chrysophyta unidentified | 2.5 | 8.8 | 7.1 | 7.1 |
| | | | *Chrysidalis peritaphrena* Schill. | 1.6 | 5.6 | 5.1 | 5.1 |
| | | | *Pterdimonas pulex* Penard. | 1.5 | 5.4 | 7.8 | 7.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 3.2 | 11.4 | 10.7 | 5.5 |
| | | | *Rhodomonas minuta* v. *nannoplanctica* Skuja | 2.6 | 9.3 | 8.4 | 4.5 |
| | | | *Cyanomonas americana* (Davis) Oltmanns | 1.7 | 6.0 | 5.7 | 3.4 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 47.7 | 61.5 | 18.5 | 12.6 |
| 77.5 (495.5) | | | *Oocystis pusilla* Hansgirg | 8.0 | 10.3 | 12.0 | 6.2 |
| | | Chrysophyta | *Sennia parvula* Skuja | 6.3 | 8.2 | 5.2 | 4.2 |
| Aug. 17, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 50.0 | 51.1 | 15.8 | 9.4 |
| 97.0 (623.2) | | | *Ctenocladus circinnatus* Borzi | 6.2 | 6.4 | 7.8 | 7.5 |
| | | Chrysophyta | *Sennia parvula* Skuja | 11.2 | 11.6 | 5.2 | 4.2 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 15.3 | 15.7 | 10.7 | 5.5 |
| | | | *Katablepharia ovalis* Skuja | 5.1 | 5.2 | 7.3 | 4.3 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 81.9 | 48.4 | 18.5 | 12.6 |
| 169.3 (477.7) | | Chrysophyta | *Pteridomonas pulex* Penard. | 29.7 | 17.6 | 7.8 | 7.0 |
| | | | *Chrysidalis peritaphrena* Schill. | 12.2 | 7.2 | 5.1 | 5.1 |
| | | | *Oochromonas globosa* Skuja | 11.4 | 6.7 | 7.6 | 7.6 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 16.4 | 9.7 | 10.7 | 5.5 |
| Aug. 23, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 19.7 | 31.2 | 15.8 | 9.4 |
| 63.2 (239.8) | | | *Chlamydomonas snowii* Printz | 7.7 | 12.1 | 14.2 | 10.7 |
| | | Chrysophyta | *Sennia parvula* Skuja | 9.1 | 14.4 | 5.2 | 4.2 |
| | | | *Chrysapsis sagene* Pasch. | 4.0 | 6.3 | 3.3 | 3.3 |
| | | | *Chrysidalis peritaphrena* Schill. | 3.4 | 5.4 | 5.1 | 5.1 |
| | | | *Heterochromonas globosa* Skuja | 3.2 | 5.1 | 8.8 | 8.8 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 9.0 | 14.2 | 10.7 | 5.5 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 43.2 | 19.8 | 18.5 | 12.6 |
| 218.0 (395.9) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 73.1 | 33.5 | 5.1 | 5.1 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 64.0 | 29.4 | 10.7 | 5.5 |
| Sept. 11, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 77.5 | 64.3 | 18.5 | 12.6 |
| 120.5 (349.8) | | Chrysophyta | *Erkenia subaequiciliata* Skuja | 15.9 | 13.2 | 4.7 | 4.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 9.6 | 8.0 | 10.7 | 5.5 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 56.1 | 28.9 | 18.5 | 12.6 |
| 194.4 (464.9) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 69.4 | 35.7 | 5.1 | 5.1 |
| | | | *Ochromonas globosa* Skuja | 14.7 | 7.6 | 7.6 | 7.6 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 29.8 | 15.3 | 10.7 | 5.5 |
| Sept. 20, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 48.7 | 25.8 | 15.8 | 9.4 |
| 188.5 (644.2) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 65.8 | 34.9 | 5.1 | 5.1 |
| | | | *Erkenia subaequiciliata* Skuja | 40.2 | 21.3 | 4.7 | 4.0 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 50.4 | 26.1 | 15.8 | 11.2 |
| 193.1 (4031.2) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 87.5 | 45.3 | 5.1 | 5.1 |
| | | | Chrysophyta unidentified | 19.0 | 9.8 | 8.0 | 7.1 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 10.7 | 5.5 | 10.7 | 5.5 |
| Oct. 4, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 103.8 | 39.2 | 15.8 | 9.4 |
| 264.9 (1127.8) | | Chrysophyta | *Erkenia subaequiciliata* Skuja | 33.1 | 12.5 | 4.7 | 4.0 |
| | | | *Chrysidalis peritaphrena* Schill. | 17.2 | 6.5 | 5.1 | 5.1 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 27.1 | 10.2 | 10.7 | 5.5 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 84.8 | 33.7 | 15.8 | 9.4 |
| 251.9 (792.0) | | | *Ctenocladus circinnatus* Borzi | 15.2 | 6.0 | 9.6 | 8.8 |
| | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 70.3 | 27.9 | 5.1 | 5.1 |
| | | | Chrysophyta unidentified | 20.4 | 8.1 | 14.9 | 10.9 |
| | | | *Pteridomonas pulex* Penard. | 12.8 | 5.1 | 7.8 | 7.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 20.3 | 8.1 | 10.7 | 5.5 |

TABLE 1. (*Continued*)

| Date | Depth | Taxa | Species | Biomass ($mg \cdot m^{-3}$) | % edible biomass | Length (μm) | Width (μm) |
|---|---|---|---|---|---|---|---|
| Oct. 18, 1989 | Epilimnion | Chlorophyta | *Oocystis borgei* Snow | 206.0 | 76.8 | 18.5 | 12.6 |
| 268.3 (554.8) | | | *Pteridomonas pulex* Penard. | 14.4 | 5.4 | 7.8 | 7.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 15.3 | 5.7 | 10.7 | 5.5 |
| | Hypolimnion | Chlorophyta | *Oocystis borgei* Snow | 193.7 | 71.1 | 18.5 | 12.6 |
| 272.4 (504.7) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 20.6 | 7.5 | 5.1 | 5.1 |
| | | | *Pteridomonas pulex* Penard. | 13.6 | 5.0 | 7.8 | 7.0 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 14.9 | 5.5 | 10.7 | 5.5 |
| Jan. 24, 1990 | Epilimnion | Chrysophyta | *Pleuromonas nastua* Skuja | 58.2 | 38.6 | 8.3 | 5.9 |
| 150.8 (236.5) | | | *Chrysidalis peritaphrena* Schill. | 43.5 | 28.8 | 5.1 | 5.1 |
| | | | *Cercobodo varians* Skuja | 29.4 | 19.5 | 11.9 | 8.0 |
| | Hypolimnion | Chrysophyta | *Pleuromonas nastua* Skuja | 14.2 | 34.7 | 8.3 | 5.9 |
| 41.0 (123.3) | | | *Chrysidalis peritaphrena* Schill. | 8.8 | 21.4 | 5.1 | 5.1 |
| | | | *Chrysochromulina parva* Lackey | 4.2 | 10.2 | 6.3 | 3.8 |
| | | | *Cercobodo varians* Skuja | 4.0 | 9.7 | 11.9 | 8.0 |
| | | | *Chrysapis sagene* Pasch. | 2.1 | 5.2 | 3.3 | 3.3 |
| | | Cryptophyta | *Rhodomonas minuta* Skuja | 3.9 | 9.5 | 10.7 | 5.5 |
| | | Euglenida | *Euglena variabilis* Klebs. | 3.3 | 8.1 | 21.4 | 8.1 |
| Feb. 28, 1990 | Epilimnion | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 12.5 | 24.2 | 5.1 | 5.1 |
| 51.7 (97.1) | | | *Cercobodo varians* Skuja | 10.3 | 19.8 | 11.9 | 8.0 |
| | | | *Pleuromonas nastua* Skuja | 4.7 | 9.0 | 8.3 | 5.9 |
| | | Euglenida | *Euglena variabilis* Klebs. | 16.3 | 31.4 | 21.4 | 8.1 |
| | Hypolimnion | Chrysophyta | *Pleuromonas nastua* Skuja | 14.1 | 38.7 | 8.3 | 5.9 |
| 36.4 (51.7) | | | *Cercobodo varians* Skuja | 12.1 | 33.3 | 11.9 | 8.0 |
| | | | *Chrysidalis peritaphrena* Schill. | 3.7 | 10.2 | 5.1 | 5.1 |
| | | Euglenida | *Euglena variabilis* Klebs. | 4.1 | 11.3 | 21.4 | 8.1 |
| Mar. 20, 1990 | Epilimnion | Chrysophyta | *Cercobodo varians* Skuja | 63.4 | 14.2 | 11.9 | 8.0 |
| 445.6 (456.9) | | | *Chrysidalis peritaphrena* Schill. | 49.4 | 11.1 | 5.1 | 5.1 |
| | | Euglenida | *Euglena variabilis* Klebs. | 258.8 | 58.1 | 17.0 | 9.5 |
| | Hypolimnion | Chrysophyta | *Cercobodo varians* Skuja | 49.3 | 39.1 | 11.9 | 8.0 |
| 126.1 (154.5) | | | *Chrysidalis peritaphrena* Schill. | 20.3 | 16.1 | 5.1 | 5.1 |
| | | | *Ochromonas globosa* Skuja | 10.3 | 8.2 | 7.6 | 7.6 |
| | | | Chrysophyta unidentified | 8.9 | 7.0 | 10.7 | 7.1 |
| | | | *Pleuromonas nastua* Skuja | 6.8 | 5.4 | 8.3 | 5.9 |
| | | Euglenida | *Euglena variabilis* Klebs. | 19.6 | 15.5 | 21.4 | 8.1 |
| May 23, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 337.9 | 70.0 | 7.5 | 4.6 |
| 482.7 (641.2) | | Chrysophyta | *Cercobodo varians* Skuja | 43.6 | 9.0 | 11.8 | 7.0 |
| | | | Chrysophyta unidentified | 27.3 | 5.6 | 13.9 | 10.2 |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 120.7 | 12.6 | 7.5 | 3.4 |
| 956.0 (1754.7) | | Chrysophyta | Chrysophyta unidentified[a] | 450.7 | 47.1 | 15.3 | 12.5 |
| | | | *Chrysidalis peritaphrena* Schill. | 176.7 | 18.5 | 6.2 | 6.2 |
| | | | *Cercobodo varians* Skuja | 65.4 | 6.8 | 11.8 | 7.0 |
| June 4, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 330.5 | 86.5 | 7.5 | 4.6 |
| 382.1 (1538.5) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 23.0 | 6.0 | 6.2 | 6.2 |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 103.0 | 14.8 | 7.5 | 4.6 |
| 696.0 (1339.5) | | Chrysophyta | *Chrysidalis peritaphrena* Schill. | 293.1 | 42.1 | 6.2 | 6.2 |
| | | | Chrysophyta unidentified | 92.3 | 13.3 | 13.5 | 10.3 |
| | | | *Pleuromonas nastua* Skuja | 60.6 | 8.7 | 9.6 | 7.0 |
| | | | *Cercobodo varians* Skuja | 46.6 | 6.7 | 11.8 | 7.0 |
| | | | Chrysophyta unidentified | 37.0 | 5.3 | 15.3 | 15.0 |
| | | | *Erkenia subaequiciliata* Skuja | 35.9 | 5.2 | 4.8 | 3.2 |
| July 3, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 237.8 | 81.3 | 7.5 | 4.6 |
| 292.6 (562.7) | | | *Oocystis borgei* Snow | 16.7 | 5.7 | 19.0 | 12.2 |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 318.0 | 52.0 | 7.5 | 4.6 |
| 611.2 (2150.5) | | Chrysophyta | *Erkenia subaequiciliata* Skuja | 125.6 | 20.5 | 4.8 | 3.2 |
| | | | Chrysophyta unidentified | 77.7 | 12.7 | 10.1 | 10.1 |
| | | | *Chrysidalis peritaphrena* Schill. | 56.2 | 9.2 | 6.2 | 6.2 |

TABLE 1. (*Concluded*)

| Date | Depth | Taxa | Species | Biomass ($mg \cdot m^{-3}$) | % edible biomass | Length (μm) | Width (μm) |
|---|---|---|---|---|---|---|---|
| Aug. 2, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 143.3 | 80.2 | 7.5 | 4.6 |
| 178.6 (287.2) | | | | | | | |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 32.8 | 11.2 | 7.5 | 4.6 |
| 292.9 (1144.5) | | Chrysophyta | *Ochromonas* sp. | 83.3 | 28.4 | 7.8 | 7.8 |
| | | | *Erkenia subaequiciliata* Skuja | 73.3 | 25.0 | 4.8 | 3.2 |
| | | | Chrysophyta unidentified | 56.4 | 19.3 | 10.4 | 10.4 |
| | | | *Chrysidalis peritaphrena* Schill. | 19.9 | 6.8 | 6.2 | 6.2 |
| | | | Chrysophyta unidentified | 15.9 | 5.4 | 7.4 | 5.5 |
| Sept. 18, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 150.6 | 84.3 | 7.5 | 4.6 |
| 178.6 (226.7) | | | *Oocystis borgei* Snow | 11.1 | 6.2 | 17.7 | 13.3 |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 160.3 | 86.1 | 7.5 | 4.6 |
| 186.1 (240.7) | | Chrysophyta | *Chrysapsis sagene* Pasch. | 13.3 | 7.2 | 5.5 | 5.5 |
| Oct. 16, 1990 | Epilimnion | Chlorophyta | *Coccomyxa* sp. | 37.3 | 54.9 | 7.5 | 4.6 |
| 68.0 (231.0) | | | *Oocystis borgei* Snow | 17.2 | 25.3 | 17.7 | 13.3 |
| | Hypolimnion | Chlorophyta | *Coccomyxa* sp. | 58.4 | 74.6 | 7.5 | 4.6 |
| 78.3 (138.9) | | Chrysophyta | Chrysophyta unidentified | 9.2 | 11.7 | 6.3 | 5.3 |
| | | | Chrysophyta unidentified | 6.7 | 8.5 | 8.9 | 6.3 |
| Jan. 3, 1991 | Epilimnion | Chlorophyta | *Tetraedron minimum* (A. Braun) Hansgirg. | 8.7 | 36.7 | 9.7 | 9.7 |
| 23.8 (137.9) | | | *Cosmarium angulosum* Breb. | 3.1 | 13.1 | 17.1 | 17.0 |
| | | | *Coccomyxa* sp. | 2.4 | 10.0 | 7.5 | 4.6 |
| | | | *Characium seiboldii* v. *parvum* Skuja | 1.5 | 6.2 | 15.1 | 5.1 |
| | | Chrysophyta | Chrysophyta unidentified | 2.8 | 11.9 | 13.0 | 7.6 |
| | | | *Chrysochromulina frigida* Holm. | 1.5 | 6.2 | 7.2 | 5.2 |
| | | Cryptophyta | *Rhodomonas minuta* v. *nannoplanctica* Skuja | 2.6 | 11.0 | 8.6 | 4.8 |
| | Hypolimnion | Chlorophyta | *Cosmarium angulosum* Breb. | 9.1 | 32.9 | 17.8 | 16.5 |
| 27.8 (311.4) | | | *Cosmarium* species | 7.4 | 26.7 | 15.5 | 15.3 |
| | | | *Oocystis borgei* Snow | 5.9 | 21.2 | 17.7 | 13.3 |
| | | | *Coccomyxa* sp. | 1.8 | 6.5 | 7.5 | 4.6 |
| | | | *Tetraedron minimum* (A. Braun) Hansgirg. | 1.5 | 5.3 | 9.7 | 9.7 |

[a]Species belonging to the order Euchromulinaceae, suborder Chromulinineae.

(Fig. 5B). Bacterial productivity was higher in the summer of 1989 compared with 1990, especially in the epilimnion (Fig 5B).

### Temporal Patterns in Total Lipid

Seasonal patterns in total lipid of *L. sicilis* differed strikingly between 1989 and 1990 (Fig. 6A). Total lipid as a proportion of dry weight ranged from 6.6 to 47.1% over the study period. In May 1989, lipid concentrations were roughly twice as high as in summer of 1989 but much lower than in spring of 1990 (Fig. 6A). Although lipid levels decreased in July of both 1989 and 1990, *L. sicilis* accumulated much greater concentrations of lipid in the summer of 1990 compared with the summer of 1989. Lipid contents increased in the fall of both years. However, in 1989, a rapid and sustained increase in lipid deposition resulted in higher winter lipid concentrations that year than in 1990 (Fig. 6A).

Seasonal patterns of total lipid content of *H. nevadensis* also differed between 1989 and 1990 (Fig. 6B). Total lipid as a proportion of dry weight ranged from 20 to 60% over the two years. Concentrations of total lipid were higher from June to September of 1990 compared with the same period in 1989 (Fig. 6B). In addition, in summer of 1989, lipid deposition was delayed compared with 1990 (Fig. 6B).

Lipid levels for *D. pulex* seldom exceeded 13% of the dry weight (Fig. 6C). Lipid deposition patterns of *D. pulex* were very different in 1989 compared with 1990. In 1989, lipid contents increased gradually from midsummer until ice formation whereas in 1990, lipid content declined rapidly during the fall (Fig. 6C).

### Temporal Patterns in Lipid Classes

In all three species, triacylglycerols were the dominant lipid class followed by phospholipids and sterols (Fig. 7). The proportion of energy reserve lipid versus structural lipid was much greater in the two copepod species compared with *D. pulex* (Fig. 7). Because triacylglycerols were the dominant and most variable lipid class, temporal patterns in triacylglycerol generally mirrored seasonal patterns in total lipids (Fig. 6 and 7).

### Empirical Models of Temporal Lipid Patterns in *L. sicilis*

Simple correlations were found between total lipid or triacylglycerol content and temperature and algal parameters, but these correlations differed with depth (Table 2). Temperature and edible algal biomass were strongly correlated with both total lipid and triacylglycerol content in the epilimnion (Table 2), negatively in the case of temperature and positively for edible algal biomass. There were weak negative correlations between total primary productivity and triacylglycerol and between total lipid and the edible portion of primary productivity in the epilimnion. The inedible fraction of the primary productivity was weakly negatively correlated with both total lipid and triacylglycerol content in the epilimnion (Table 2). Chlorophyll and the

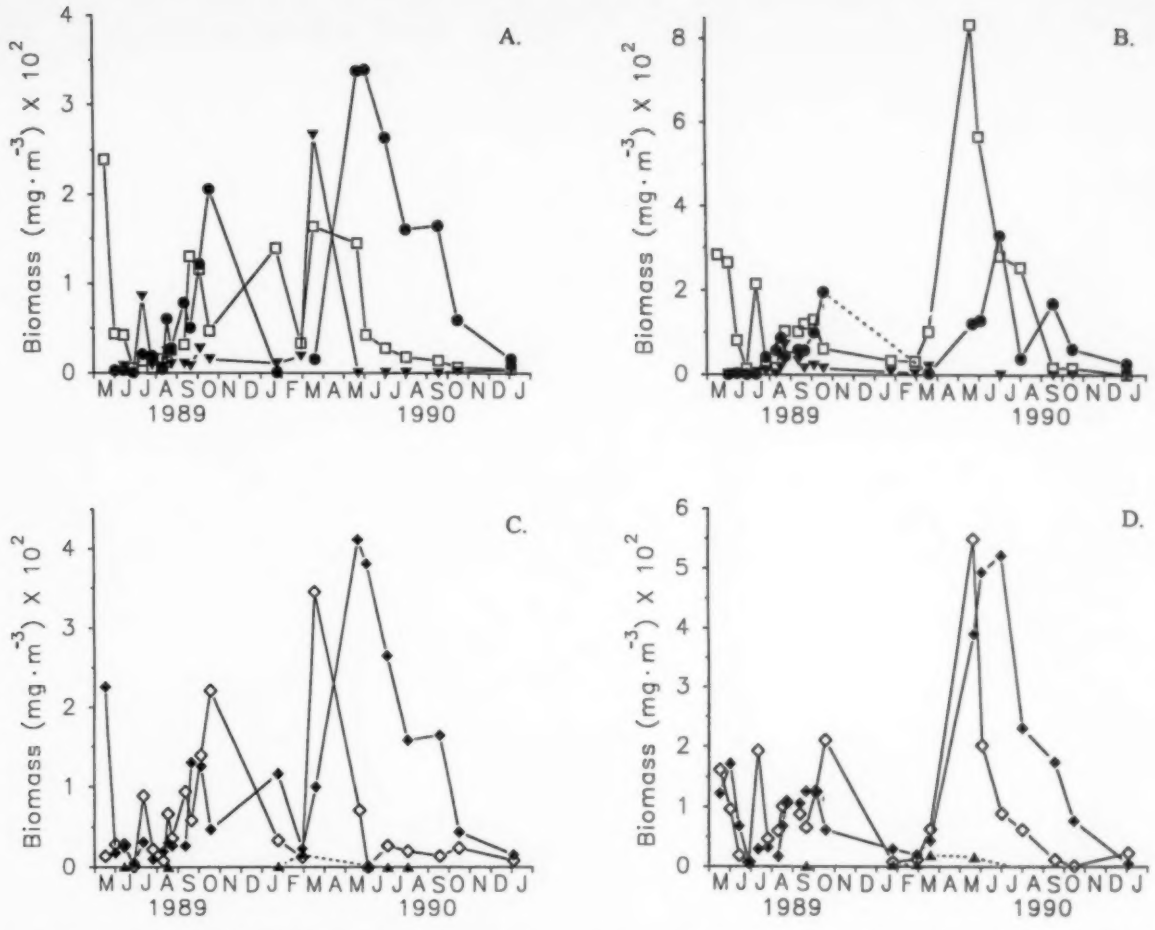



FIG. 3. Biomass of presumed edible algal taxa (A and B) and size categories (C and D) from the epilimnion (A and C) and hypolimnion (B and D) of Redberry Lake (1989–91). Chrysophyta, open squares; Chlorophyta, solid circles; Cryptophyta and Euglenidae, solid inverted triangles; 1–10 μm, solid diamonds; 10–20 μm, open diamonds; 20–40 μm, solid triangles.

edible algal biomass were significantly positively correlated to both total lipid and triacylglycerol content in the hypolimnion (Table 2) although the correlations were not strong.

The relative importance of each variable known or suspected to affect seasonal patterns in lipid content (chlorophyll, edible algal biomass, primary productivity rates, and temperature) can be better summarized by developing an empirical multivariate model that allows prediction of seasonal lipid patterns of *L. sicilis*. Stepwise multiple regression was used to isolate those variables that best explained seasonal variation in either total lipid concentration (percent lipid) or triacylglycerol content (micrograms per individual) of *L. sicilis* in Redberry Lake over the ice-free seasons of 1989 and 1990. Mean epi- and hypolimnetic water temperature and epilimnetic edible algal biomass together accounted for 92% of the total lipid content (percentage of dry weight) of *L. sicilis* (Table 3). The regression model for triacylglycerol content included mean epilimnetic temperature, epilimnetic edible algal biomass, and epilimnetic primary productivity attributable to edible algae which together explained 88% of the total variance (Table 3). Partitioning primary productivity into a separate category which approximated the edible portion of primary productivity enhanced the predictive capability of this model. Chlorophyll concentration was a poor predictor of lipid levels in this herbivore and did not enter into either regression model.

Stepwise multiple regression of measured nutrient parameters against either total lipid content or triacylglycerol content of *L. sicilis* resulted in simple regression models that had little predictive power with respect to explaining seasonal variation in these two lipid variables. Ammonia concentration was the only nutrient that was accepted ($F$ to accept = 4.0) by the forward stepwise procedure ($r^2 = 0.16$, $p = 0.04$) in relation to total lipid of *L. sicilis*. Similarly, mean oxygen content of the water column was weakly associated with triacylglycerol content of *L. sicilis* ($r^2 = 0.24$, $p = 0.01$)

## Discussion

### *Leptodiaptomus sicilis*

Lipid reserves of zooplankton, in lakes that are ice-covered for a portion of the year, are often high initially, despite low food

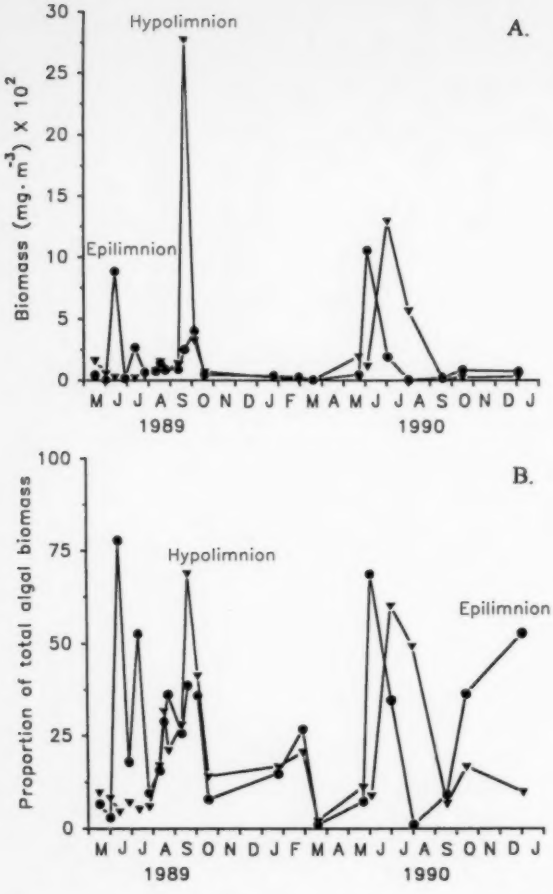

Hypolimnion
A.
Biomass (mg · m$^{-3}$) X 10$^2$
Epilimnion
M J J A S O N D J F M A M J J A S O N D J
1989
1990
B.
Proportion of total algal biomass
Hypolimnion
Epilimnion
M J J A S O N D J F M A M J J A S O N D J
1989
1990

FIG. 4. (A) Total diatom biomass and (B) proportion of total algal biomass represented by diatoms from the epi- and hypolimnion of Redberry Lake.

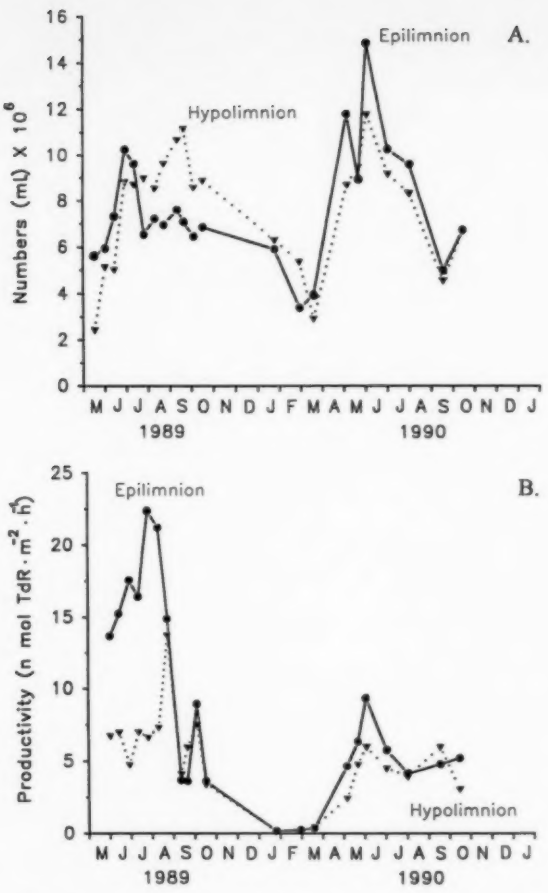

Epilimnion
A.
Numbers (ml) X 10$^6$
Hypolimnion
M J J A S O N D J F M A M J J A S O N D J
1989
1990
B.
Productivity (n mol TdR · m$^{-2}$ · h$^{-1}$)
Epilimnion
Hypolimnion
M J J A S O N D J F M A M J J A S O N D J
1989
1990

FIG. 5. Time trajectories of (A) bacterial numbers and (B) bacterial productivity for the epi- and hypolimnion of Redberry Lake (1989–90).

levels, and then gradually decline, due to metabolic losses (see also Arts et al. 1992). This is particularly true of Redberry Lake where under-ice water often supercools to as low as −0.5°C by January or February. Thus, in the following sections, we focus only on periods during the ice-free season when lipid concentrations of *L. sicilis* were relatively high or low to infer nutritional suitability of particular taxa, size classes, and species.

Lipid deposition and loss rates depend primarily on respiration rates (a function of temperature) and on food quality and quantity. In deepwater offshore regions of Lake Michigan, *L. sicilis* migrates down to deeper, colder water during the warm summer months (Vanderploeg et al. 1992). Utilization of this "thermal refuge" provides *L. sicilis* with a relatively stable food supply and reduced metabolic rates at depth, thus facilitating an accelerated rate of lipid deposition (Vanderploeg et al. 1992). Redberry Lake develops a strong thermocline at about 8 m during summer. We did not find any evidence that *L. sicilis* populations were concentrated at depth in Redberry Lake (M.S. Evans, personal observation), suggesting that individuals migrated freely between the epi- and hypolimnion. This raises the possibility that some proportion of the *L. sicilis* population in Redberry Lake can, at any given time, make trade-offs between deeper cooler water, where respiration rates are lower, and warmer surface waters depending on the availability of edible algae in these two depth strata. The mean water temperature for the hypolimnion of Redberry Lake was warmer in late summer of 1990 compared with 1989 (Fig. 1), and these were also differences in food quantity and quality between the two depth zones, particularly in 1990 (see below). For these reasons, we decided to partition the temperature and algal data between the epi- and hypolimnion and treat the means of these parameters as separate variables in the regression models.

Periods of high or low lipid content of *L. sicilis* (Fig. 6A) tracked chlorophyll *a* concentrations (Fig. 2A) over most of the ice-free season in both years, with the exception of spring 1989 when chlorophyll *a* was very high but lipid levels were modest and fall of 1990 when chlorophyll concentrations were modest but lipid levels were unusually high. The inability of total chlorophyll concentrations to consistently predict lipid levels of *L. sicilis* is not surprising given that inedible forms often dominated the algal biomass. Size fractionation of phytoplankton by filtration to remove larger cells and colonies prior to chlorophyll analyses might be useful in improving the correlations between zooplankton energy reserve lipid and chlorophyll concentration.

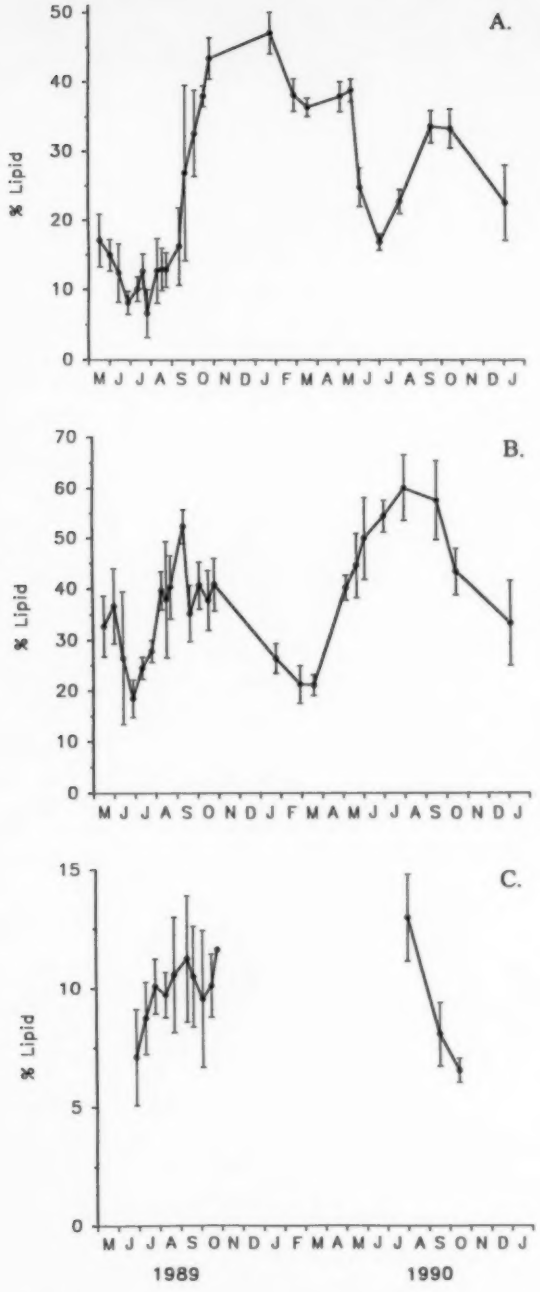



FIG. 6. Seasonal lipid patterns as a percentage of dry weight of (A) *L. sicilis*, (B) *H. nevadensis* and (C) *D. pulex* in Redberry Lake (1989–91). Vertical bars are 95% confidence intervals. *Daphnia pulex* is not found in Redberry Lake from January to July.

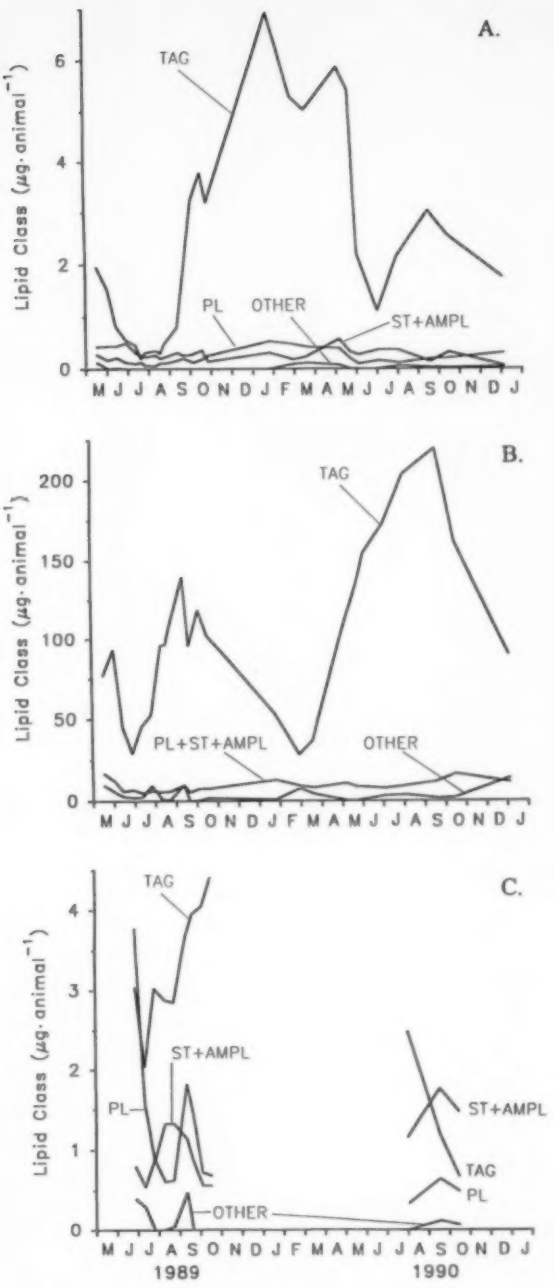



FIG. 7. Seasonal patterns of major lipid classes of (A) *L. sicilis*, (B) *H. nevadensis* and, (C) *D. pulex* in Redberry Lake (1989–91). TAG = triacylglycerols, PL = polar lipids, ST = sterols, AMPL = acetone-mobile polar lipids. OTHER comprises free fatty acids and trace amounts of wax esters, methyl esters, and hydrocarbons.

Edible algal biomass was high in spring and fall of 1989 and spring and early summer of 1990 (Fig. 2B) when lipid levels of *L. sicilis* were also high (Fig. 6A). A similar correlation between edible algal biomass and neutral lipid levels (all zooplankton species combined) was demonstrated by Wainman and Lean (1990). If *L. sicilis* were to take advantage of the accentuated metabolic gains brought about by feeding in relatively cooler water, we would expect to observe a large, sustained increase in lipid deposition. In agreement, total lipid concentrations and triacylglycerol contents of *L. sicilis* were much higher during the

hypolimnetic edible algal maxima in May and June of 1990 than during the same period in 1989 (Fig. 6A and 7A). Total lipid deposition and triacylglycerol content (Fig. 6A and 7A) increased in the fall when water temperatures (Fig 1) were decreasing quickly and edible algal biomass was moderately high (Fig. 2A). Winter lipid levels appeared to be dependent on the amount of lipid accumulated the preceding fall. Hence, fall edible algal biomass was greater in 1989 compared with 1990, and subsequent midwinter lipid levels (1990) of *L. sicilis* reflected this difference (Fig. 6A).

Since dietary transfer of free fatty acids from phytoplankton to herbivorous zooplankton occurs with little modification (Goulden and Place 1990), and relatively quickly, there exists a potential that lipid energy reserves can, under certain circumstances, be used to infer nutritional suitability of wild populations of particular algal species to herbivorous zooplankton species. The ability to infer nutritional suitability of particular algal species or groups of similar sized species should be enhanced in Redberry Lake, since the food web is structurally simple. In May 1989, lipid concentrations of *L. sicilis* were roughly twice as high as in summer of 1989 (Fig. 6A). Small chrysophytes (<20 μm) dominated the edible algal biomass (Fig. 3). Thus, it appears likely that *C. varians*, *C. nitens*, and especially *C. peritaphrena* (Table 1) were primarily responsible for the higher lipid values of *L. sicilis* in May and early June 1989 compared with midsummer. Conversely, poor nutritional quality can be ascribed to *P. gelatinosa* and *C. planctonica* which together comprised 55% of the total biomass from the hypolimnion during midsummer of 1989. The large diatom *S. niagarae* can also be considered nutritionally inadequate or unavailable, since it comprised 65.5% of the total algal standing crop in the epilimnion on June 14, a time when lipid levels of *L. sicilis* were very low.

The large increase in lipid concentration in *L. sicilis* in fall of 1989 appears to have been related to a succession of small (1–20 μm) species consisting of chlorophytes, chrysophytes, and a cryptophyte (Fig. 3). The primary species implicated as being responsible for the increased lipid deposition of *L. sicilis* during this period include the chlorophyte *O. borgei*, the chrysophyte *C. peritaphrena*, and the cryptophyte *R. minuta* (Table 1). The appearance of *R. minuta* has been shown to be correlated with rapid increases in triacylglycerol of *L. sicilis* in a nearby hypereutrophic lake (Arts et al. 1992).

Lipid levels of *L. sicilis* were very high in May and June of 1990. One or more chrysophyte and/or chlorophyte species between 1 and 20 μm (Fig. 3) are associated with the relatively high lipid concentrations observed in *L. sicilis* during this period (Fig. 6A and 7A). Despite increasing water temperatures, lipid contents of *L. sicilis* started to increase in June. The presence of small chlorophytes such as *Coccomyxa* sp. and chrysophytes such as *C. peritaphrena*, *C. varians*, and *E. subaequiciliata* and other chrysophytes belonging to the order Chromulinineae was associated with this increase in lipid and may therefore be of high nutritional quality for *L. sicilis* (Table 1). As the water temperature continued to increase (Fig. 1), lipid levels decreased to a summer low in July 1990 even though the biomass of *Coccomyxa* sp. was high at this time. This may have occurred because many of the surviving adults of *L. sicilis* were spent after the June reproductive period and also because many of the other edible species that were abundant in May and June (e.g., *C. peritaphrena*) were no longer as abundant (Table 1). Despite this decline, lipid levels of *L. sicilis* remained higher in July of 1990 compared with July of 1989 (Fig. 6A and 7A), likely a result of the consistently higher biomass of edible algae in 1990 (Table 1; Fig. 3C).

In August and September of 1990, lipid levels again increased (Fig. 6A and 7A). Edible algal species at this time were dominated by the small spherical green algae *Coccomyxa* sp. (Table 1). Although some species of *Coccomyxa* are known to be colonial and/or covered by a mucilaginous sheath (Prescott 1982), we observed only single-celled nonencapsulated forms. The sustained increase in lipids during the fall of 1990 at a time when *Coccomyxa* clearly dominated the assemblage of presumed edible algal species suggests that the species of *Coccomyxa* found in Redberry Lake is of relatively high quality as a food resource for *L. sicilis*. It is likely that the combination of high rates of primary productivity (Fig. 2C), decreasing temperatures (Fig. 1), and modest edible biomass levels (Fig. 2B) produced the increase in lipid deposition observed in August and September of 1990 (Fig. 6A and 7A).

### Empirical Models of Temporal Lipid Patterns of *L. sicilis*

Simple correlations amongst temperature or algal parameters showed that mean epilimnetic temperature and the edible fraction of total algal biomass were significantly correlated with both total lipid and triacylglycerol content (Table 2). The multiple regression procedure selected combinations of variables that best explained seasonal patterns in either total lipid or triacylglycerol content. Not surprisingly, epilimnetic temperature and edible algal biomass were included in both models (Table 3). Although weakly correlated with both total lipid and triacylglycerol in the hypolimnion by simple correlation (Table 2), chlorophyll was rejected by the stepwise regression criteria (Table 3). The lack of predictive power of chlorophyll with respect to lipid content of herbivorous zooplankton is consistent with previous results obtained in a nearby eutrophic lake (Arts et al. 1992).

The combined effects of three of the variables selected by the stepwise regression procedure on lipid content of *L. sicilis* were visualized using a three-dimensional representation (Fig. 8) constructed from the regression equation for the ice-free seasons of both years. These figures are partial solutions because only low and high edible algal concentrations are modelled. The model makes several generalizations with respect to the effects of epi- and hypolimnetic temperature and epilimnetic edible algal biomass on lipid content. An increase in mean epilimnetic temperature is associated with a decrease in lipid content whereas an increase in mean hypolimnetic temperature is associated with an increase in lipid content. Lipid contents are excepted to increase by roughly 20% between the lower (100 $mg \cdot m^{-3}$) and upper (600 $mg \cdot m^{-3}$) boundaries of epilimnetic edible algal biomass for any given combination of mean epi- and hypolimnetic temperatures (Fig. 8). These boundaries roughly mimic the low and high end of the concentration of edible algae observed in Redberry Lake over the 2-yr observation period, although a single peak of edible algae greater that 600 $mg \cdot m^{-3}$ was observed in the hypolimnion at the end of May 1990 (Fig. 2B). Thus, a sustained increase in edible algal biomass in near surface waters can offset the effects of warm temperatures in the epilimnion.

Hypolimnetic edible algae biomass was not accepted by the stepwise regression procedure. However, inspection of seasonal trends in edible algal biomass (Fig. 2B) suggest that, with the exception of late May to early June 1990 when hypolimnetic biomass was substantially higher than epilimnetic biomass, overall seasonal patterns of epi- and hypolimnetic edible

TABLE 2. Pearson's correlation coefficients amongst total lipid, triacylglycerol, temperature, and selected phytoplankton parameters for two depth strata in Redberry Lake over the ice-free seasons of 1989–90 ($n = 20$). Total lipid and triacylglycerol values are for the entire water column. The blank rows separate the epilimnetic variables from the hypolimnetic variables. TLIP = Total lipid, TAG = triacylglycerol, TB = total algal biomass, EB = edible algal biomass, TP = total primary productivity for the entire water column, EP = edible fraction of primary productivity, IP = inedible fraction of primary productivity, $*P \leq 0.05$, $**P \leq 0.001$.

| | | | Epilimnion | | | | | | | Hypolimnion | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TLIP | TAG | Temp. | Chl | TB | EB | TP | EP | IP | Temp. | Chl. | TB | EB | EP | IP |
| TLIP | 1.00 | 0.93** | –0.78** | 0.43 | 0.05 | 0.75** | –0.44 | 0.44* | –0.55* | 0.15 | 0.47* | 0.23 | 0.50* | 0.17 | –0.23 |
| TAG | | 1.00 | –0.82** | 0.31 | 0.05 | 0.83** | –0.54* | 0.37 | –0.54* | –0.15 | 0.48* | 0.19 | 0.63* | 0.12 | –0.24 |
| Temp. | | | 1.00 | –0.41 | –0.03 | –0.57** | 0.68** | –0.14 | 0.52* | 0.26 | –0.51* | –0.09 | –0.38 | 0.03 | 0.27 |
| Chl | | | | 1.00 | 0.19 | 0.25 | –0.10 | 0.02 | –0.26 | 0.16 | 0.74** | 0.64* | 0.02 | –0.11 | 0.17 |
| TB | | | | | 1.00 | 0.35 | –0.09 | –0.46* | –0.30 | –0.28 | 0.33 | 0.23 | 0.29 | –0.21 | –0.21 |
| EB | | | | | | 1.00 | 0.35 | 0.23 | –0.60* | –0.26 | 0.59* | 0.42 | 0.89** | 0.16 | –0.14 |
| TP | | | | | | | 1.00 | 0.07 | 0.63* | 0.41 | –0.36 | –0.20 | –0.50* | 0.15 | 0.19 |
| EP | | | | | | | | 1.00 | –0.04 | 0.56* | –0.04 | –0.08 | 0.04 | 0.55* | 0.13 |
| IP | | | | | | | | | 1.00 | 0.11 | –0.46* | –0.36 | –0.62* | –0.01 | 0.46* |
| Temp. | | | | | | | | | | 1.00 | –0.24 | –0.04 | –0.39 | 0.38 | 0.23 |
| Chl | | | | | | | | | | | 1.00 | 0.77** | 0.50* | –0.10 | 0.03 |
| TB | | | | | | | | | | | | 1.00 | 0.42 | –0.24 | 0.09 |
| EB | | | | | | | | | | | | | 1.00 | 0.22 | –0.09 |
| EP | | | | | | | | | | | | | | 1.00 | 0.53* |
| IP | | | | | | | | | | | | | | | 1.00 |

biomass in the two depth horizons were similar, especially given that only a single sample was counted from each depth horizon on each sampling date. Thus, edible algae biomass in either depth horizon could probably be used interchangeably in the model for most of the 2-yr observation period. The model suggests that small (2–3°C) increases in mean hypolimnetic temperature within the 1–10°C range could result in significant gains in lipid for those animals that choose to feed at depth. The tilt of the planes (downwards to the right) suggests that a greater concentration of edible algal biomass is needed in the epilimnion compared with the hypolimnion to produce the same gain in lipid content (Fig. 8). For example, an extrapolation from the model suggests that it would take ≈600 mg·m$^{-3}$ of edible algal biomass to maintain lipid levels of *L. sicilis* at ≈20% when the mean epilimnetic temperature is at 21°C. However, *L. sicilis* could sustain the same lipid content (20%) by feeding in the hypolimnion (at ≈6–8°C) with only ≈100 mg · m$^{-3}$ of available edible algal biomass (Fig. 8). This reinforces the thermal refuge concept in that, with respect to lipid deposition, it would be advantageous for *L. sicilis* to feed when algae of high nutritional quality are available at depth. In support of this, lipid contents of *L. sicilis* were very high in early summer of 1990 (Fig. 6A) when epi- and especially hypolimnetic edible biomass peaked (Fig. 2B).

### *Hesperodiaptomus nevadensis*

Maximum lipid levels (60% of dry weight) of *H. nevadensis* recorded here are similar to maximum lipid contents of *H. nevadensis* reported by Hairston (1979) from two saline lakes in Washington State and among the highest levels reported for freshwater or marine copepods. For example, Sargent and Henderson (1986) summarized lipid contents of 10 genera comprising 28 different species of marine copepods, and only three of these species had lipid levels in excess of 60% (range 61–74%). The high lipid contents of *H. nevadensis* are comparable with the freshwater "glacial relict" calanoid *Limnocalanus macrurus*, although that species' energy reserves are primarily in the form of wax esters (Cavaletto et al. 1989).

Experimental studies involving *H. nevadensis* have shown that this copepod is primarily carnivorous, preferring rotifers and juvenile copepods, especially copepodites of *L. sicilis*, with which it frequently co-occurs (Anderson 1970; Hairston 1979). When adult *H. nevadensis* were offered mixed prey assemblages, which included *D. pulex*, only copepod prey were eaten (Anderson 1970). The size of the feeding basket (range 0.46 – 0.62 mm; $\bar{x} = 0.54 \pm 0.05$ mm) formed by the mouthparts of adult *H. nevadensis* in Redberry Lake defines the maximum prey size of juvenile *L. sicilis* likely consumed by *H. nevadensis*. Adults of *L. sicilis* are usually larger than 0.6 mm in Redberry Lake and probably exceed the gape size of *H. nevadensis*. Therefore, copepodites of *L. sicilis*, especially C3–C5's (range ≈0.4–0.6 mm), are probably most vulnerable to predation by *H. nevadensis*. In 1989, C3–C5 copepodites of *L. sicilis* were most abundant around the middle of August, but in 1990 these stages were already abundant in early July (M.S. Evans, personal observation). These differences in the timing of the appearance of C3–C5 copepodites of *L. sicilis* are reflected in the time course of lipid deposition in *H. nevadensis* in 1989 versus 1990 (Fig. 6B and 7B). For example, by July 1990, lipid levels of *H. nevadensis* had already reached ≈50% of the dry weight whereas in July 1989, lipid levels were only at ≈25% (Fig. 6B).

The movement of lipid from *L. sicilis* to *H. nevadensis* can be envisaged as a direct predator–prey energy transfer. The relative simplicity of the food web in Redberry Lake allows us to see this transfer more clearly than in more complex food webs where other interactions (e.g., other predators, competitors) diversify the number of pathways that the energy can take. Our data suggest that unusually high lipid levels observed in *L. sicilis*

TABLE 3. Physical and biological variables in relation to total and energy reserve lipid content identified by forward stepwise regression ($F$ value to enter = 4). EP = the proportion of the primary productivity estimated to be due to edible algal biomass in the epilimnion.

| Model ($r^2$) | Dependent variable | Coefficient | SE | $t$-value | Significance level |
|---|---|---|---|---|---|
| Total lipid (0.92) | Constant | 22.11 | 3.47 | 6.37 | <0.001 |
| | Epilimnetic temperature | −1.21 | 0.16 | −7.34 | <0.001 |
| | Epilimnetic edible biomass | 0.04 | 0.01 | 6.30 | <0.001 |
| | Hypolimnetic temperature | 2.01 | 0.31 | 6.40 | <0.001 |
| Triacylglycerol (0.88) | Constant | 2.74 | 0.62 | 4.46 | <0.001 |
| | Epilimnetic temperature | −0.16 | 0.03 | −5.16 | <0.001 |
| | Epilimnetic edible biomass | 0.04 | 0.001 | 4.93 | <0.001 |
| | | | EP | | |

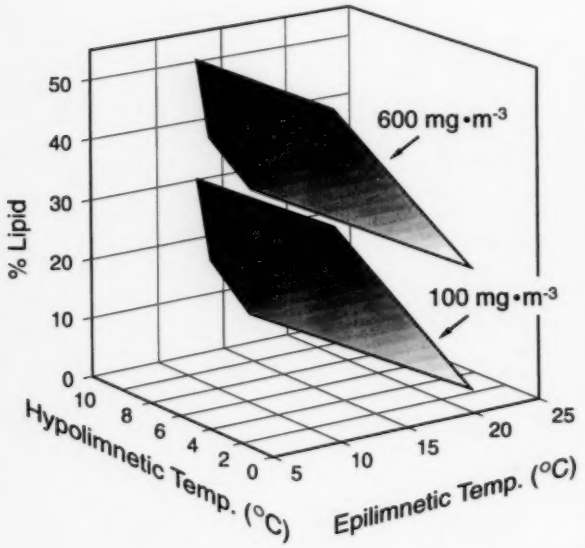



FIG. 8. Representation of a multiple regression model showing predicted lipid contents of *L. sicilis* as a function of mean epi- and hypolimnetic water temperatures at high and low epilimnetic edible algal biomass concentrations in Redberry Lake. Note that portions of each plane are truncated in order to satisfy the constraint that mean epilimnetic temperatures must be greater than mean hypolimnetic temperatures.

during the spring of 1990 as a result of an abundance, especially at depth, of small chlorophytes and chrysophytes were ultimately responsible for the high lipid levels observed in *H. nevadensis* during summer and early fall of 1990. Because the size of the feeding basket limited *H. nevadensis* to consuming copepodites of *L. sicilis*, the gains in lipid energy reserves acquired by *L. sicilis* in early spring were not fully transferred to *H. nevadensis* until middle to late summer (Fig. 6A, 6B, 7A, and 7B). Thus, physical constraints imposed by the size of the feeding basket of *H. nevadensis* provide a mechanistic hypothesis to explain time lags in this simple predator–prey energy transfer.

*Daphnia pulex*

The seasonal patterns of total lipid content and triacylglycerol lipid of *D. pulex* in 1989 and 1990 tracked patterns in chlorophyll *a* and total edible phytoplankton biomass. In the fall of 1989, chlorophyll and edible algae increased until October (Fig. 2A and 2B) concomitant with a gradual increase in total lipid and triacylglycerol content (Fig. 6C and 7C). In the fall of 1990, by contrast, chlorophyll remained low and edible algal biomass declined steadily throughout the fall. This was reflected in declining total lipid contents and triacylglycerol levels of *D. pulex* (Fig. 6C and 7C). During the fall, there was little difference in chlorophyll or total edible algal biomass between the epi- and hypolimnion.

Small (1–10 μm) to intermediate (10–20 μm) sized algae dominated the edible algal biomass in late summer and fall of both years although biomass was greater in the fall of 1989 (Fig. 3). Chrysophyta and chlorophyta were the dominant edible taxa during both fall periods (Fig. 3). Cryptophyta (primarily *R. minuta*) were much more abundant in mid-summer and fall of 1989 than in 1990 (Table 1). Chrysophytes were represented by a variety of species, but *C. peritaphrena* and *E. subaequiciliata* were the most common and abundant. Chlorophytes, which likely contributed to the gains in lipids observed in *D. pulex* 1989, were represented by *Oocystis* sp. In August of 1990, when *D. pulex* lipid contents were still relatively high (Fig. 6C), *Coccomyxa* sp. were very abundant, but lipid contents declined when *Coccomyxa* sp. biomass fell in September and October (Table 1). Thus, the same suite of species implicated in the seasonal lipid patterns of *L. sicilis* appear to be responsible for the seasonal lipid patterns observed in *D. pulex*.

Although bacteria are not usually considered high sources of dietary lipid, indirect transfer of carbon from bacteria to zooplankton via protistan grazers may contribute to higher lipid deposition rates when bacteria are very abundant and cladocerans dominate (Wylie and Currie 1991; Arts et al. 1992). *Daphnia* can, however, consume bacteria, particularly if the bacteria are large (Lampert 1987). Redberry Lake bacteria are relatively large; coccoid cells varied in size from monthly mean diameters of 0.43 μm in winter to 0.56 μm in spring, while rods had a mean width of 0.35 μm (range 0.16–0.57 μm) and a mean length of 1.37 μm (range 0.97–2.02 μm) (Tumber et al. 1993). The mesh opening formed by the feeding appendages of *Daphnia* sp. typically exceed 0.2 μm. For example, Geller and Müller (1981) reported that mesh openings from five common *Daphnia* sp. ranged from 0.23 to 1.8 μm, while Lampert (1987) summarized data on *D. pulex* showing a mean mesh size of between 0.40 and 0.51 μm. Although bacterial sizes in Redberry Lake overlap with published values of mesh size opening of *Daphnia* sp., bacterial numbers in fall of 1989 versus 1990 were similar and could not account for differences in the lipid depo-

sition patterns observed in these two years (Fig. 5A). Similarly, bacterial productivity rates were comparable from August to October in both years (Fig. 5B). Thus, it seems unlikely that bacteria play a major role in determining lipid deposition seasonal patterns of *D. pulex* in this saline lake. This observation cannot be generalized to other saline lakes because of the paucity of similar data on *Daphnia* nutrition, bacterial volumes, biomass, and productivity.

Goulden and Place (1990) stated that lipid contents of daphniid Cladocera are generally between 20 and 30% of their dry mass. In laboratory feeding experiments, they demonstrated that lipid contents of *D. pulex* can range from 19.8 to 40% depending on the instar and food level. Lipid contents of field populations of *D. pulex* from a nearby eutrophic lake ranged from 10 to 40% with the low concentrations corresponding to a period of low edible algal biomass (Arts et al. 1992). Unlike nearby Humboldt Lake where *D. pulex* persists year-round (Arts et al. 1992), the appearance of *D. pulex* in Redberry Lake is restricted to the midsummer to fall period. By comparison, lipid contents of *D. pulex* in hypertrophic Humboldt Lake reached 30% by late fall and 40% of dry weight over the winter (Arts et al. 1992). The low lipid contents (7–13%) and unusual life history strategy of *D. pulex* in Redberry Lake suggest that the species has been pushed into a subsistence niche by increasing salinity levels.

Based on historical data, Van Stempvoort et al. (1993) have shown that Redberry Lake levels have dropped from 512 m (above mean sea level) in the early 1900's to somewhat less than 504 m (above mean sea level) at present. Salinity (as total dissolved solids) has increased from $\approx 11$ g · L$^{-1}$ in the 1920's to 22.8 g · L$^{-1}$ in the hypolimnion in June of 1991 (Van Stempvoort et al. 1993). Concomitantly, pelagic zooplankton species diversity has decreased in Redberry Lake compared with 1940 (Moore 1952). Notably absent today are the cladocerans *Daphnia longispina, Diaphanosoma leuchtenbergianum*, and *Ceriodaphnia quadrangula* reported by Moore in 1952. In lakes and ponds, *D. pulex* are either found throughout the year or else usually appear soon after ice-off and are then present all summer long. In Redberry Lake, *D. pulex* typically appears in July and persists until ice formation. This strategy is unusual and may be a unique adaptation to living in a saline system. In the spring, salinity decreases with additions of relatively fresh water from runoff and ice-melt. Over the summer, salinity gradually increases primarily as a result of evaporative losses. It is well know that "freezing out" of freshwater as surface ice forms results in an additional increase in salinity of the underlying water (Hammer 1990). In addition to the osmotic stress imposed by increased salinity under ice, zooplankton must cope with effects of supercooling due to the freezing point depression typical of salt solutions. Supercooling further limits the number of species that can persist under the ice. This is because membrane fluidity, critical to the maintenance of cell function, depends on fatty acid composition and temperature (Farkas et al. 1984).

Climatic warming, if it occurs as predicted, may accentuate evaporation rates and further increase the salinity of lakes, especially in areas with endorheic drainage (Hammer 1990). Hammer (1990) reported a maximum salinity tolerance for *D. pulex* of 30 g · L$^{-1}$, based on a collection from Spain. Aladin (1991) reported that all Cladocera disappeared from the Aral Sea when the salinity reached 30–32 g · L$^{-1}$. Given the present trend in increasing salinity, it appears likely that *D. pulex* will eventually vanish from Redberry Lake. When exactly *Daphnia* will be eliminated is difficult to predict although a simple extrapolation based on the current trend (Hammer 1990) suggests that salinity will exceed 30 g · L$^{-1}$ sometime between the years 2010 and 2020. However, the different ion proportions, especially the high sulphate concentrations of Redberry Lake compared with the saline lakes of Spain and the Aral Sea, will affect predictions of time-to-extinction as a function of salt concentration.

## Acknowledgements

We are indebted to W.S. Gardner and J.F Cavaletto (National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Ann Arbor) for providing initial guidance on the microgravimetric and Iatroscan methodologies. J.C. Earle provided expert identification and quantification of the seasonal changes in phytoplankton species. E. Marles, P. Shaw, and M. Waiser provided technical assistance. The manuscript benefited from suggestions made by W.S. Gardner and H.A. Vanderploeg and two anonymous reviewers. This research was supported by a Canadian Government Laboratory Visiting Fellowship to M.T.A. and by the National Hydrology Research Institute (M.T.A., R.D.R., and M.S.E.).

## References

ALADIN, N.V. 1991. Salinity tolerance and morphology of the osmoregulation organs in Cladocera with special reference to Cladocera from the Aral Sea. Hydrobiologia 225: 291–299.

ANDERSON, R.S. 1970. Predator–prey relationships and predation rates for crustacean zooplankters from some lakes in western Canada. Can. J. Zool. 48: 1229–1240.

ARTS, M.T., M.S. EVANS, AND R.D. ROBARTS. 1992. Seasonal patterns of total and energy reserve lipids of dominant zooplanktonic crustaceans from a hyper-eutrophic lake. Oecologia 90: 560–571.

BOURDIER, G.G., AND C.A. AMBLARD. 1989. Lipids in *Acanthodiaptomus denticornis* during starvation and fed on three different algae. J. Plankton Res. 11: 1201–1212.

CARPENTER, S.R., AND J.F. KITCHELL. 1992. Trophic cascade and biomanipulation: interface of research and management — A reply to the comment by DeMelo et al. Limnol. Oceanogr. 37: 208–213.

CAVALETTO, J.F., H.A. VANDERPLOEG, AND W.S. GARDNER. 1989. Wax esters in two species of freshwater zooplankton. Limnol. Oceanogr. 34: 785–789.

CHOW-FRASER, P. 1985. Herbivorous feeding of zooplankton in lakes of south central Ontario. Ph.D. thesis, University of Toronto, Toronto, Ont. 96 p.

CUHEL, R.L., P.B. ORTNER, AND D.R.S. LEAN. 1984. Night synthesis of protein by algae. Limnol. Oceanogr. 19: 731–744.

EARLE, J.C., H.C. DUTHIE, AND D.A. SCRUTON. 1987. Factors influencing the distribution of phytoplankton in 97 headwater lakes in insular Newfoundland. Can. J. Fish. Aquat. Sci. 44: 639–649.

FARKAS, T., G.Y. NEMECZ, AND I. CSENGERI. 1984 Differential response of lipid metabolism and membrane physical state by an actively and passively overwintering planktonic crustacean. Lipids 19: 436–442.

FOLCH, J., M. LEES, AND G.H.S. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

GARDNER, W.S., W.A. FREZ, E.A. CICHOCKI, AND C.C. PARRISH. 1985. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. 30: 1099–1105.

GELLER, W., AND H. MÜLLER. 1981. The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia 49: 316–321.

GOULDEN, C.E., AND A.R. PLACE. 1990. Fatty acid synthesis and accumulation rate in daphnids. J. Exp. Zool. 256: 168–178.

HAIRSTON, N.G. JR. 1979. The adaptive significance of color polymorphism in two species of *Diaptomus* (Copepoda). Limnol. Oceanogr. 24: 15–37.

HAMMER, U.T. 1978. The saline lakes of Saskatchewan. III. Chemical characterization. Int. Rev. Gesamten Hydrobiol. 63: 311–335.

HAMMER, U.T. 1986. Saline lake ecosystems of the world. Dr. W. Junk Publ., Dordrecht, The Netherlands. 616 p.

HAMMER, U.T. 1990. The effects of climate change on the salinity, water levels and biota of Canadian prairie saline lakes. Verh. Int. Ver. Limnol. 24: 321–326.

HAMMER, U.T., AND R.C. HAYNES. 1978. The saline lakes of Saskatchewan II. Locale, hydrography and other physical aspects. Int. Rev. Gesamten Hydrobiol. 63: 179–203.

HAMMER, U.T., AND J.M. HESELTINE. 1988. Aquatic macrophytes in saline lakes of the Canadian prairies. Hydrobiologia 158: 101–116.

HAMMER, U.T., J. SHAMESS, AND R.C. HAYNES. 1983. The distribution and abundance of algae in saline lakes of Saskatchewan, Canada. Hydrobiologia 105: 1–26.

HAMMER, U.T., J.S. SHEARD, AND J. KRANABETTER. 1990. Distribution and abundance of littoral benthic fauna in Canadian prairie saline lakes. Hydrobiologia 197: 173–192.

HAYNES, R.C., AND U.T. HAMMER. 1978. The saline lakes of Saskatchewan. IV. Primary production by phytoplankton in selected saline ecosystems. Int. Rev. Gesamten Hydrobiol. 63: 337–351.

LAMPERT, W. 1987. Feeding and nutrition in *Daphnia*, *In* R.H. Peters and R. de Bernardi [ed.] *Daphnia*. Memorie Dell'Istituto Italiano di Idrobiologia Dott. Marco de Marchi. Vol. 45. Consiglio Nazionale Delle Recerche. Verbania Pallanza. 502 p.

MOORE, J.E. 1952. The Entomostraca of southern Saskatchewan. Can. J. Zool. 30: 410–450.

NUSCH, E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Arch. Hydrobiol. Beih. Ergebn. Limnol. 14: 14–36.

PARRISH, C.C. 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44: 722–731.

PORTER, K.G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature (Lond.) 244: 179–180.

PRESCOTT, G.W. 1982. Algae of the western great lakes. Otto Koeltz Science Publ., Koenigstein, Germany. 977 p.

RAWSON, D.S., AND J.E. MOORE. 1944. The saline lakes of Saskatchewan. Can. J. Res. D 22: 141–201.

REYNOLDS, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge. p. 196–197.

ROBARTS, R.D., M.S. EVANS, AND M.T. ARTS. 1992. Light, nutrients and water temperature as determinants of phytoplankton production in two saline, prairie lakes with high sulphate concentrations. Can. J. Fish. Aquat. Sci. 49: 2281–2290.

ROBARTS, R.D., AND L.M. SEPHTON. 1981. The enumeration of aquatic bacteria using DAPI. J. Limnol. Soc. S. Afr. 7: 72–74.

ROBARTS, R.D., AND R.J. WICKS. 1989. [Methyl-$^3$H]thymidine macromolecular incorporation and lipid labelling: their significance to DNA labelling during measurements of aquatic bacterial growth rate. Limnol. Oceanogr. 34: 213–222.

ROSS, P.E., AND M. MUNAWAR. 1981. Preference for nanoplankton size fractions in Lake Ontario zooplankton grazing. J. Great Lakes Res. 7: 65–67.

SARGENT, J.R., AND R.J. HENDERSON. 1986. Lipids, p. 59–108. *In* E.D.S. Corner and S.C.M. O'Hara [ed.] The biological chemistry of marine copepods. Clarendon Press, Oxford.

SAUNDERS, G.E., F.B. TRAMA, AND R.W. BACHMAN. 1962. Evaluation of a modified $^{14}$C technique for shipboard estimation of photosynthesis in large lakes. Publ. Great Lakes Res. Div. 8: 61 p.

SHIFRIN, N.S., AND S.W. CHISHOLM. 1981. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light–dark cycles. J. Phycol. 17: 374–384.

SPRULES, W.G., AND J.E. BOWERMAN. 1988. Omnivory and food chain length in zooplankton food webs. Ecology 69: 418–426.

STERNER, R.W., D.G. HAGEMEIER, R.F. SMITH, AND W.L. SMITH. 1992. Lipid–ovary indices in food limited *Daphnia*. J. Plankton Res. 14: 1449–1460.

TESSIER, A.J., L.L. HENRY, C.E. GOULDEN, AND M.W. DURAND. 1983. Starvation in *Daphnia*. Energy reserves and reproductive allocation. Limnol. Oceanogr. 28: 667–676.

TIMMS, B.V., U.T. HAMMER, AND J.W. SHEARD. 1986. A study of benthic communities in some saline lakes in Saskatchewan and Alberta, Canada. Int. Rev. Gesamten. Hydrobiol. 71: 759–777.

TUMBER, V.P., R.D. ROBARTS, M.T. ARTS, M.S. EVANS, AND D.E. CALDWELL. 1993. The influence of environmental factors on seasonal changes in bacterial cell volume in two prairie saline lakes. Microb. Ecol. 25. (In press)

VANDERPLOEG, H.A. 1988. *Diaptomus* vs. net phytoplankton: effects of algal size and morphology on selectivity of a behaviourally flexible, omnivorous copepod. Bull. Mar. Sci. 43: 377–394.

VANDERPLOEG, H.A. 1990. Feeding mechanisms and particle selection in suspension–feeding zooplankton, p. 184–208. *In* R.S. Wotton [ed.] The biology of particles in aquatic systems. CRC Press Inc., Boca Raton, Fla.

VANDERPLOEG, H.A., W.S. GARDNER, C.C. PARRISH, J.R. LIEBIG, AND J.F. CAVALETTO. 1992. Lipids and life-cycle strategy of a hypolimnetic copepod in Lake Michigan. Limnol. Oceanogr. 37: 413–424.

VAN STEMPVOORT, D.R., T.W.D. EDWARDS, M.S. EVANS, AND W.M. LAST. 1993. Palaeoenvironmental studies in a saline prairie lake, southern Saskatchewan, Canada, using mineralogy, stable isotopes and pigments. J. Palaeontol. (In press)

WAINMAN, B.C., AND D.R.S. LEAN. 1990. Seasonal trends in planktonic lipid content and lipid class. Ver. Int. Ver. Limnol. 24: 416–419.

WYLIE, J.L., AND D.J. CURRIE. 1991. The relative importance of bacteria and algae as food sources for crustacean zooplankton. Limnol. Oceanogr. 36: 708–728.

# Estimation of Effective Effort from Catch-at-Age Data

J.E. Paloheimo and Yong Chen

*Department of Zoology, University of Toronto, Toronto, ON M5S 1A1, Canada*

Paloheimo, J.E., and Y. Chen. 1993. Estimation of effective effort from catch-at-age data. Can. J. Fish. Aquat. Sci. 50: 2421–2428.

We present a method for estimating effective efforts or fishing mortality rates based on a linearized version of the catch equation. Catch-at-age for at least two age groups over a series of years is required. The method presupposes a value for natural mortality rate ($M$). The method is validated using simulated data with an appropriate error structure. The algorithm always converges to a set of effective efforts that are compatible with the known catches. Nevertheless, the solution to the basic equations is not unique although the different solutions are typically highly correlated. If the $M$ assumed by the algorithm is the same as the actual $M$ the iterated effective efforts are typically very close to the true effective efforts or fishing mortality rates. If the assumed $M$ is too high or too low the pattern of effective efforts is still recovered to a high degree of accuracy, typically $0.90 < r < 1.00$, even though $M$ may be off by as much as 60%. When data for three or more age groups are available the method is extended to at least squares procedure that takes into account the increasing uncertainty of catches with age.

Les auteurs présentent une méthode pour évaluer les efforts réels ou les taux de mortalité par pêche fondée sur une version linéarisée de l'équation des prises. Il faut obtenir les données de capture selon l'âge pour au moins deux groupes d'âge pendant un certain nombre d'années. La méthode présuppose une valeur pour le taux de mortalité naturelle ($M$). La méthode est validée par des données de simulation avec une structure appropriée d'erreur. L'algorithme converge toujours vers un ensemble d'efforts réels compatibles avec les captures connues. Pourtant, la solution aux équations de base n'est pas unique même si les différentes solutions sont en général fortement corrélées. Si la valeur $M$ hypothétique de l'algorithme est la même que la valeur $M$ réelle, les efforts effectifs répétés sont en général très proches des efforts efficaces vrais ou du taux de mortalité par pêche. Si la valeur $M$ hypothétique est trop élevée ou trop basse, le profil des efforts réels est encore retrouvé avec un niveau élevé de précision, en général $0{,}90 < r < 1{,}00$, même si l'écart de la valeur $M$ peut atteindre 60 %. Lorsqu'il existe des données pour les trois groupes d'âge ou plus la méthode est élargie à une méthode des moindres carrés qui tient compte de l'incertitude accrue des captures avec l'âge.

*Received November 24, 1992*
*Accepted May 31, 1993*
*(JB696)*

*Reçu le 24 novembre 1992*
*Accepté le 31 mai 1993*

A simple model of the process of fishing or exploitation of wildlife leads to what is generally called the catch equation. The model considers exploitation as a random sampling with removal. This leads to a linear relationship between the effort and the instantaneous mortality generated by it; the proportionality constant is called the catchability coefficient. This may indeed be the case for fisheries that exploit more or less randomly distributed stocks or that do not have access to modern searching devices such as echo sounders or fish finders. However, back in 1964, Paloheimo and Dickie (1964) had already suggested that the relationship between effort and fishing mortality may not be so simple. Analysis of catch and effort data combined with tagging studies of Georges Bank haddock, (*Melanogrammus aeglefinus*) by Crecco and Overholz (1990) confirmed the suggestions by Paloheimo and Dickie for that fishery.

Both the possibility of a complex relationship between effort and the size of the stock or cohort and the uncertainties and difficulties of measuring the effort in the first place led many authors to consider the determination of fishing mortality independent of effort data. The first and perhaps still most common method of doing so is called cohort analysis (Pope 1972). Cohort analysis gives not only the estimates of fishing mortalities but also cohort sizes at entry to the fishery. The drawback of cohort analysis is that the natural mortality and the terminal fishing mortality (fishing mortality at the last age) for each cohort have to be assumed. Some variant of these assumptions were retained by all subsequent refinements of Pope's cohort analysis by Doubleday (1976), Fournier and Archibald (1982), Deriso et al. (1985), and others.

Pope and Sheperd (1982) developed an algorithm to generate fishing mortalities based on catch-at-age data given three parameters: (1) the value of the natural mortality rate, (2) the fishing mortality rate at the final year, and (3) what they called the exploitation pattern at the last age. In addition, they assumed that the pattern of fishing could be factored into the age and year effects. This is called the separability assumption.

The method presented here has many similarities with that by Pope and Shepherd (1982). For one thing, we need to assume a value for the natural mortality rate ($M$) and also make some assumptions about the fishing mortality rate. The assumption that we have chosen is that the average decline of log catch-at-age from one age to the next is an estimate of the average total mortality rate, corrected if necessary for differences in selectivities at age. Alternatively, we could have made the same assumption as Pope and Shepherd did. The assumption that we chose is in our view weaker, since it is asymptotically correct as the number of years increases. We also make the separability assumption unless data exist to the contrary.

The basic differences between the two methods are that our

method is based on a linearized version of the catch equation and that the resultant equations are simple and show clearly the effect of various parameters such as selectivities on the estimates of the fishing mortality. The algorithm to solve them can be implemented on a spreadsheet program such as Microsoft's *Excel* or *Lotus 1-2-3*.

Because of the simplicity of the basic equations the variance–covariance matrix of log catches can be worked out. This allows us to apply a least squares procedure to estimate annual fishing mortality rates based on the individual data points rather than just on the marginal totals. By including the variance–covariance matrix as a part of the algorithm, we can take into account not only the covariance between the estimated mortalities but also the increasing uncertainty about the catches of older age groups.

## Basic Notation

Let $O_{ij}$ = observed catch-at-age $i$ year $j$, $C_{ij}$ = actual/observed catch-at-age $i$ year $j$, $E_j$ = effective effort in year $j$ for fully recruited age groups, $M$ = natural mortality rate, and $Me$ = assumed natural mortality rate. For ease of following the notation, we will use $C_{ij}$ for observed catches except in the section (Error Structure) where we need to distinguish between the observed and actual catches. There, $O_{ij}$'s are used for observed catches and $C_{ij}$'s for the actual catches. We will not assume that $E_j$'s are known. However, we do assume that $E_{ij}$ = effective effort at age $i$ year $j$ can be written as

$$E_{ij} = s_i E_j$$

where $s_i$ is the gear selectivity at age $i$. This is typically called the separability assumption. Later on, we consider an example where this assumption is relaxed.

Whenever required, we will assume that $q$, the catchability coefficient, has the value 1, $q = 1$. We can fix the value of $q$ arbitrarily, since it is a scaling factor dependent on the units in which effort is measured. By setting it as 1 the effective effort will equal the fishing mortality rate.

Most importantly, we assume that the basic catch equation is valid. Using the linearized version of it (Paloheimo 1961, 1980), we can write

$$(1) \quad y_{ij} = \ln C_{ij} = \ln(E_j) + \ln(s_i q) + \ln(N_{ij}) - \tfrac{1}{2} M - \tfrac{1}{2} s_i q E_j .$$

This equation is an approximation. To be more exact, the multiplier 1/2 should be modified depending on the value of the total mortality $Z$ where $Z = M + s_i q E_j$. For $Z = 0.1, 0.3, 0.5, 0.7$, and 0.9 the multiplier should be 0.50 (exact), 0.49, 0.48, 0.47, and 0.46 instead of the global 0.5. However, the error of approximation relative to the typical variability in fisheries data is small enough to be safely ignored.

### Error Structure

The observed catches at age $O_{ij}$ differ from the actual catches because the total catch is an estimate and because the fraction of that catch that are $i$-yr-olds is also an estimate. We can write

$$O_{ij} = O_j P_{ij}$$

where $O_j$ is the estimate of the actual catch $C_j$ and $P_{ij}$ is the estimated fraction of the $i$-yr-olds. If the sampling for ages is random then the variance of $P_{ij}$ is approximately $P_{ij}(1 - P_{ij})/n$ where $n$ is the sample size. The covariance between $P_{ij}$ and $P_{i'j'}$ is $-P_{ij} P_{i'j'}/n$ when $j = j'$ and 0 otherwise.

The relative catchabilities at age may also vary from year to year. If we incorporate the relative catchability with the gear selectivity and denote the age and year specific catchabilities by $s_{ij}$ the catch Eq. 1 should actually be written as

$$(1') \quad \ln C_{ij} = \ln(E_j) + \ln(s_{ij} q) + \ln(N_{ij}) - \tfrac{1}{2} M - \tfrac{1}{2} s_{ij} q E_j$$

where $s_{ij}$ is the relative catchability at age $i$ and year $j$. Note that while the overall catchability is also most likely to vary from year to year (e.g., due to changing hydrological conditions), this does not effect our error structure in the current paper because any such variations in $q$ can be absorbed in our estimate of the effective effort.

In simulating the data, we first generate, somewhat arbitrarily, the annual effective efforts and the cohort sizes at the beginning of the first year of their entry into our database. We next generate $s_{ij}$'s by assuming that they are log normally distributed. More specifically, we put

$$s_{ij} = s_i e^{\Delta s_{ij}} \text{ where } \Delta s_{ij} \in N(0, \sigma_{ss}) .$$

We can now calculate the catches $C_{ij}$ and the total catch $C_j = \Sigma_i C_{ij}$. The observed catch is then generated from

$$O_j = C_j e^{\Delta C_j} \text{ where } \Delta C_j \in N(0, \sigma_{cc}) .$$

As a last step, binomial sampling of $O_j$'s could be carried out with probabilities $P_{ij}$ given by $P_{ij} = C_{ij}/C_j$. This presupposes that estimation of the total catch is more or less independent of the composition of the catch. The binomial sampling has not been incorporated in the present paper.

The level of error and the magnitude of fluctuations can now be specified by fixing $\sigma_{ss}$ and $\sigma_{cc}$. Here, we refer to their levels as 10% error, 20% error, and so on where 20% (e.g.) has been calculated from

$$1.2 = e^{2\sigma} .$$

That is, the 20% "error" implies that with probability 0.95 the error is less than 20%. Because the errors act exponentially the fluctuations in downward direction are somewhat less, in this case 17% instead of 20% (1/1.2 = 0.83). That is, the errors in catchabilities and total catches all have a somewhat skewed distribution.

## Estimation of Effective Effort

The first step in the analysis is to carry out a two-way ANOVA of pairwise mortalities at age and year (Shepherd and Nicholson 1991). If the mortalities between years are significant, we may proceed further. If the mortalities between years are not significant, it may be because they are not in fact significant in which case a simpler analysis that presupposes constant effective effort and total mortality can be carried out. Alternatively, they may not be significant because the separability assumption is not valid. We may still estimate the effective efforts for each pair of age groups and scrutinize the results for any obvious patterns.

Two Age Groups

Consider first the two age groups only, say ages 1 and 2. For the time being, we assume that $s_i$'s are known. They can be estimated only if more than two age groups are considered at a time. It follows from Eq. 1 that the log ratio of catches is given by

$$(2)\quad z_{12j} = \ln E_j - \ln E_{j+1} + \ln s_1 - \ln s_2 + \frac{q}{2}(s_1 E_j + s_2 E_{j+1}) + M .$$

Assuming that $s_i$' are known, there are now altogether $n + 1$ unknowns, $E_1,\ldots, E_{n+1}$, not including $q$ and $M$. The value of $q$ can be arbitrarily set, since the effective efforts are at best determined only up to a multiplicative constant in any case. The natural mortality rate we choose is our best guess, say $M = 0.2$. We will need one more equation to determine $E_j$'s.

We now assume that the average of $z_{12j}$ over all years equals the average mortality rate, corrected for selectivities if different at ages 1 and 2. Even with this additional constraint, there does not seem to be a unique solution to Eq. 2. The true values of $E_i$'s satisfy them but so do many others. However, the following algorithm seems to give us rather quickly a convergent set of effective efforts that satisfy Eq. 2 and are highly correlated with the actual efforts in simulated data.

Let $E_{kj}$ be the value of $E_j$ at the $k$th iteration. We take $q = 1$ and set $M$ at some fixed value. We begin the iteration by taking as the initial values

$$(3)\quad E_{1j} = [\bar{Z} - M - \ln s_1 + \ln s_2]/[q(s_1 + s_2)/2]$$

where

$$\bar{Z} = \sum_{j=1}^{n} z_{12j}/n .$$

The subsequent iterations are obtained by setting

$$E_{k+1,j} = E_{kj}(1 + \Delta E_{kj})$$

where we put $\Sigma\Delta E_{kj} = 0$. This ensures that the total mortality is $\bar{Z}$ in Eq. 3 at each iteration.

Rewriting Eq. 2 for the $k$th iteration and making use of the approximation $\ln(1 + \Delta E_{kj}) = \Delta E_{kj}$, we get

$$(4)\quad \Delta z_{12j} = (1 + \tfrac{1}{2} q\, s_1 E_{kj})\,\Delta E_{kj} + (-1 + \tfrac{1}{2} q\, s_2 E_{k,j+1})\,\Delta E_{kj+1}$$

$$(4')\quad 0 = \sum_j \Delta E_{kj}$$

where $\Delta z_{12j} = \Delta z_{12j} - \ln s_1 + \ln s_2 - \mathrm{M} - \ln E_{kj} + \ln E_{kj+1} - \frac{1}{2} q\,(s_1 E_{kj} + s_2 E_{kj+1})$, i.e., $\Delta z_{12j}$'s (no subscript for iteration) are the residual mortalities at the $k$th iteration. Leaving out the subscript for the order of the iteration above, this may be written in matrix notation as

$$(5)\quad \Delta\mathbf{z} = \begin{pmatrix} a_1 & b_1 & 0 & \ldots & 0 & 0 \\ 0 & a_2 & b_2 & \ldots & 0 & 0 \\ . & . & . & \ldots & . & . \\ 0 & 0 & 0 & \ldots & a_n & b_n \\ 1 & 1 & 1 & \ldots & 1 & 1 \end{pmatrix} \Delta\mathbf{E}$$

where $\Delta\mathbf{z}'$ (transpose of $\Delta\mathbf{z}$) $= (\Delta z_{11}, \ldots, \Delta z_{1n}, 0)$, $\Delta\mathbf{E}' = (\Delta E_{k1}, \ldots, \Delta E_{kn+1})$, $a_j = 1 + \frac{1}{2} q s_1 E_{kj}$, and $b_j = -1 + \frac{1}{2} q s_2 E_{kj+1}$. Equation 5 is a linear equation and can readily be solved on any spreadsheet program that has a matrix inversion capability.

To examine the effect of our constraint, Eq. 4', we assume for simplicity that $s_1 = s_2 = 1$. In this case, we start our iterations with all the effective efforts set at $(\bar{Z} - M)/q$. By insisting that $\Sigma\Delta E_{kj} = 0$, we assume that $\bar{Z}$ is an estimate of the average total mortality rate over all years, a reasonable assumption, since $\bar{Z}$ is in fact the observed average total mortality rate. Its value can be calculated from Eq. 2, giving

$$\bar{Z} = \frac{1}{n}(\ln E_1 - \ln E_{n+1}) + q\,\bar{E} + M$$

where $\bar{E} = \frac{1}{n}(\frac{1}{2}E_1 + E_2 + \ldots + E_n + \frac{1}{2}E_{n+1})$. The sum of the last two terms above, $q\bar{E} + M$, may be considered as the average total mortality rate. We note that $\bar{Z}$ is a biased estimate of it, the magnitude of the bias being $\frac{1}{n}(\ln E_1 - \ln E_{n+1})$. In many cases the bias is small and has a small effect on our estimate. This is especially true if the number of years is large.

The assumption that the average decline in log catch-at-age is an estimate of the average total mortality rate is not essential. Other assumptions such as those made by Pope and Shepherd (1982) can easily be accommodated. We need only to modify Eq. 4' suitably. To fix the effort in the last year of the fishery, we would write Eq. 4' as $0 = \Delta E_{k,n+1}$. To implement the modifications in Eq. 5, only the last row of the $n + 1 \times n + 1$ matrix would need to change. The last row would now be replaced by (0,...,0,1) (Pope and Shepherd's case). In this case, successive iterations would lead to estimates identical to the ones given by the Pope and Shepherd algorithm.

Use of an incorrect $M$ has a predictable effect. If our guess for $M$ is incorrect the level of estimated efforts will be affected but the estimated efforts will still be highly correlated with the actual efforts.

Figure 1 shows a typical situation. Data have been generated using the catch equation and the known efforts ($E$) and population sizes ($N$) ($E$: 5, 3, 2, 3, 4, 3, 5, 4, 3, 4, 4; $N$: 1100, 800, 700, 500, 400, 200, 600, 900, 100, 700). In this case the data are exact. Figure 1a shows what happens if we assume a correct value of $M$, in this case, $M = 0.20$. The iterative procedure does not completely recover the exact values of the effective effort even though the data are determined by the catch equation without any sampling or process errors. Yet the agreement is quite respectable ($r^2 = 0.97$).

The lack of recovery of exact values is not due to our use of the approximate linearized catch equation. The estimated effort values are always consistent with the input catches even though they may have been generated using different effort data.

When the assumed natural mortality rate is in error, say $Me = 0.3$ instead of the correct value, $M = 0.2$ in Fig. 1b, or $Me = 0.2$ instead of the correct value, $M = 0.3$ in Fig. 1c, the method still recovers the pattern of effective effort. The correlation between the estimated effective effort and the true effort is quite high ($r^2 = 0.95$, Fig. 1b; $r^2 = 0.97$, Fig. 1c).

The estimated effective effort for the last year in the data series is the least accurate compared with the other years' estimates. This is expected, since the effort for the last year appears only once in all the equations (e.g., Eq. 4), while the efforts for all the other years, with the exception of the first, appear twice. This is further illustrated below.

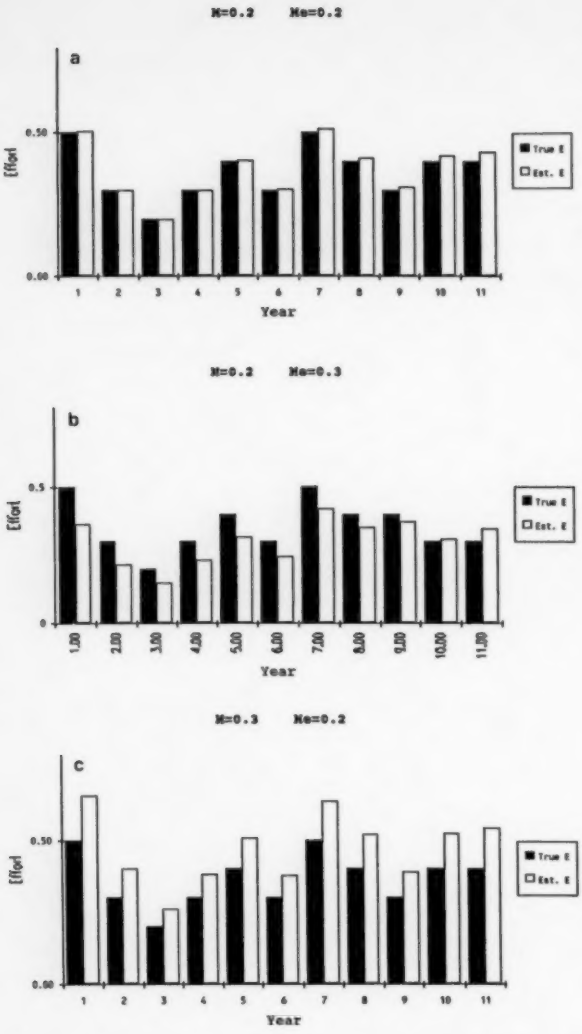



FIG. 1. Estimated and true efforts for a combination of the natural mortality rate $M$ and assumed mortality rate $Me$. (a) $M = 0.2$ and $Me = 0.2$; (b) $M = 0.2$ and $Me = 0.3$; (c) $M = 0.3$ and $Me = 0.2$.

To test the method in a variety of circumstances, we ran six sets of 100 runs each by generating both the efforts and cohort sizes randomly. In the first three sets the catches were calculated assuming a 20% variability in age-specific catchabilities. The natural mortality was set at $M = 0.25$ and the guessed value of $M$ set at $Me = 0.25$ (Set 1), $Me = 0.15$ (Set 2), and $Me = 0.4$ (Set 3). In the second series of three, we imposed an additional 20% error on the observed catches and repeated the natural mortality patterns. The exact procedure is set out in Table 1.

The performance of the algorithm is shown in Table 2 which shows the histogram of correlation coefficients between the actual and estimated efforts done separately for each of the 100 runs. Surprisingly the correlations are uniformly quite high even though the mortality rate used by the algorithm may be quite incorrect ($Me = 0.4$ or 0.15 instead of $M = 0.25$). The outliers, that is the runs that have a low correlation, occur when the difference between the first and last effort is relatively high. This is demonstrated in Fig. 2 which corresponds to the first set of runs in Table 1. In Fig. 2, we have plotted the difference between the last actual effective effort and the first on the $y$-axis and the corresponding correlation coefficient on the $x$-axis. By and large, low correlations occur only when the difference is extreme.

Somewhat different correlations are shown in Table 3. Here, we looked at each year separately for all 100 runs and correlated the estimated and actual efforts. The correlations are uniformly high irrespective of whether the natural mortality rate is correct or not. The only exception occurs for the last year in the data sets when the correlation is almost nonexistent.

Even though we may have a high correlation between the estimated and the actual effort, there may still be systematic bias. This is shown in Fig. 3 where we have plotted the estimated effort against the actual effort. The data are the same as in Fig. 1c. The line shown on the graph is the diagonal. If the estimated efforts are the same as the actual efforts the points should lie on the diagonal. It turns out that not only are the actual points above the line (this is because we used an incorrect $Me < M$), but also the line drawn through them has a slope that is different from 1, in this case 1.32. This means that the high efforts are overestimated more than the low efforts. By and large, if the natural mortality rate is underestimated, i.e., $Me < M$, the slope tends to be greater than 1. If the natural mortality rate is overestimated, i.e., $Me > M$, the slope is typically less than 1. In this case the high efforts are underestimated relative to the low efforts.

## Combined Estimates

When the annual effective efforts are estimated for an individual age pair the estimates subsume not only the variability at age (as they should) but also all the errors of sampling as well. To shed some light on the extent to which the estimated annual changes in the effective efforts by pairs of ages are true for the fishable stock as a whole, we need a combined estimate.

We now have $a$ pairs of ages. The algorithm is more or less the same as it would be for a single pair with an exception that we now have far more data points than unknowns and the algorithm must be amended to include a least squares procedure.

The initial starting values given in Eq. 3 are more or less the same with the obvious change that

$$\bar{Z} = \sum_{i=1}^{a} \sum_{j=1}^{n} z_{i,j+1,j} / an .$$

To show how Eq. 5 needs to change, we denote the upper $n \times (n+1)$ matrix of the right-hand side by $\mathbf{A}$:

$$(6) \quad \mathbf{A} = \begin{pmatrix} a_1 & b_1 & 0 & \dots & 0 & 0 \\ 0 & a_2 & b_2 & \dots & 0 & 0 \\ . & . & . & \dots & . & . \\ 0 & 0 & 0 & \dots & a_n & b_n \end{pmatrix}.$$

Let $\Delta \mathbf{z}' = (\Delta z_{121}, \dots, \Delta z_{12n}, \Delta z_{231}, \dots, \Delta z_{a,a+1,n}, 0)$ and $\Delta \mathbf{E}'$ as before. Also, let $(1)'$ stand for a row vector of 1's, i.e., $(1)' = (1,\dots,1)$. Then, if we have four age groups,

TABLE 1. Pattern of generating data for testing the algorithm. All random numbers $e_i$, $e_i$, and $e_j$ are normal, $N(0,1)$. $M$ is the value used in the catch equation and $Me$ is the value used in the recovery algorithm.

| Set | $f_i$ | $N_j$ | $M$ | $Me$ | Error in catch (%) | Error in catchability (%) |
|---|---|---|---|---|---|---|
| 1 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.25 | 0 | 20 |
| 2 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.15 | 0 | 20 |
| 3 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.40 | 0 | 20 |
| 4 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.25 | 20 | 20 |
| 5 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.15 | 20 | 20 |
| 6 | $4e^{-0.5e_i}$ | $500e^{e_j}$ | 0.25 | 0.40 | 20 | 20 |

TABLE 2. Correlation coefficients between the actual and estimated efforts for the data sets in Table 1.

| | Set | | | | | |
|---|---|---|---|---|---|---|
| $r$ | 1 | 2 | 3 | 4 | 5 | 6 |
| 0.9–1.00 | 71 | 72 | 72 | 66 | 64 | 63 |
| 0.8–0.9 | 16 | 16 | 15 | 21 | 23 | 21 |
| 0.7–0.8 | 8 | 7 | 5 | 7 | 7 | 9 |
| 0.0–0.7 | 5 | 5 | 8 | 6 | 6 | 7 |

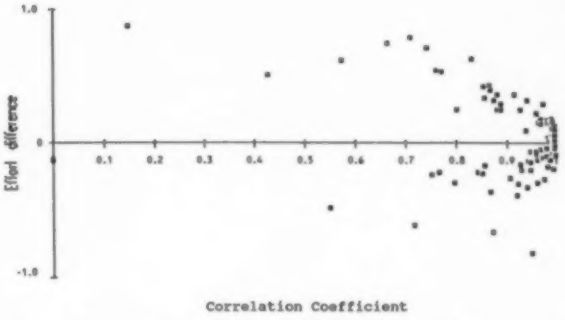



FIG. 2. Difference between the first and the last effort plotted against the correlation coefficient between the estimated and actual efforts.

$$(7)\quad \Delta \mathbf{z} = \begin{pmatrix} A_1 \\ A_2 \\ A_3 \\ (1)' \end{pmatrix} \Delta \mathbf{E} = \mathbf{B}\,\Delta \mathbf{E}$$

where $\mathbf{A}_1$, $\mathbf{A}_2$, and $\mathbf{A}_3$ all have the same structure as $\mathbf{A}$ in Eq. 6. The elements of $\mathbf{A}_1$ are calculated using the selectivities $s_1$ and $s_2$, those of $\mathbf{A}_2$ are calculated using the selectivities $s_2$ and $s_3$, and so on.

In Eq. 7, there are $3n + 1$ equations and only $n + 1$ unknowns. To estimate them, we take a recourse to a least squares procedure giving

$$(8)\quad \Delta \mathbf{E} = (\mathbf{B}'\mathbf{V}^{-1}\mathbf{B})^{-1}\mathbf{B}'\mathbf{V}^{-1}\Delta \mathbf{z}$$

where $\mathbf{V}$ denotes an appropriate variance–covariance matrix. This is given in the Appendix.

Alternatively, we can get a combined estimate of efforts by averaging the estimates obtained separately for each age pair. This, as shown below, is better than first getting the marginal totals of $z_{i,i+1,j}$'s over the ages ($i$) and then subjecting the marginal averages to the algorithm for two age groups as suggested by Pope and Shepherd (1982) (along with a different algorithm).

Generalized least squares estimates, estimates based on the marginal totals, and estimates based on the average of separate estimates for each age pair were compared using six data sets, simulated with the same initial conditions as in Table 1 for four age groups. Only 10 runs were made at each setting. The results for data set 1 are shown in Fig. 4 where we have plotted the average difference between the true and estimated efforts. The other data sets gave qualitatively similar results.

From Fig. 4, it appears that by and large the least squares procedure seems to give the best results, although the advantage over the average of the separate estimates is relatively small. The estimates based on marginal totals have invariably the largest bias. In real data the error variance typically increases with age. Since this can be taken into account in the variance–covariance matrix $\mathbf{V}$, the least squares estimates are expected to be superior to estimates based on the individual values of $z_{i,i+1,j}$'s or on their marginal totals.

## Estimating Selectivities

We have so far assumed that all $s_i$'s have known values. If this is not the case, they may be estimated if $M$ is indeed constant for all ages. We found that it is better to estimate the selectivities and effective efforts separately rather than simultaneously. As the simultaneous estimation procedure is quite complicated and often fails to give reasonable estimates even at moderate error levels, we will not go into it further.

To estimate the selectivities, we sum Eq. 2 over all years for each age pair. With four age groups, we get

$$(9)\quad \begin{aligned} \bar{z}_{12} &= \ln s_1 - \ln s_2 + \tfrac{1}{2} q\,(s_1 + s_2)\,\bar{E} + M \\ \bar{z}_{23} &= \ln s_2 - \ln s_3 + \tfrac{1}{2} q\,(s_2 + s_3)\,\bar{E} + M \\ \bar{z}_{34} &= \ln s_3 - \ln s_4 + \tfrac{1}{2} q\,(s_3 + s_4)\,\bar{E} + M \end{aligned}$$

where we have ignored the term $\frac{1}{n}(\ln E_1 - \ln E_{n+1})$.

The above equations show how observed average mortality rates are affected by the selectivities. If we know two of them, say $s_3 = s_4 = 1$, the other two can be solved by an iterative procedure that is similar to the one used to solve for $E_j$'s.

TABLE 3. Correlation coefficients between the actual and estimated efforts by years over all 100 runs. Initial conditions as in Table 1.

| | Year | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Set | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1 | 1.00 | 1.00 | 1.00 | 0.99 | 0.99 | 0.98 | 0.97 | 0.96 | 0.93 | 0.93 | 0.00 |
| 2 | 0.99 | 1.00 | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 0.97 | 0.94 | 0.92 | −0.03 |
| 3 | 0.99 | 0.98 | 0.97 | 0.96 | 0.96 | 0.96 | 0.92 | 0.92 | 0.89 | 0.93 | 0.04 |
| 4 | 0.97 | 0.97 | 0.94 | 0.94 | 0.95 | 0.94 | 0.93 | 0.96 | 0.88 | 0.91 | 0.09 |
| 5 | 0.97 | 0.97 | 0.94 | 0.95 | 0.96 | 0.95 | 0.96 | 0.95 | 0.91 | 0.91 | 0.01 |
| 6 | 0.93 | 0.94 | 0.89 | 0.90 | 0.91 | 0.90 | 0.88 | 0.90 | 0.84 | 0.91 | 0.06 |

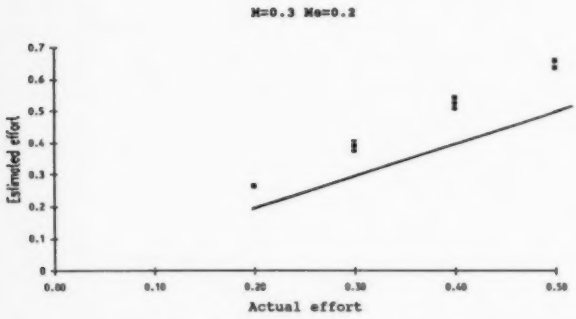



FIG. 3. Relationship between the actual and estimated effort when the assumed natural mortality rate is less than the actual rate. Data are the same as in Fig. 1c. A diagonal line is drawn for comparison.

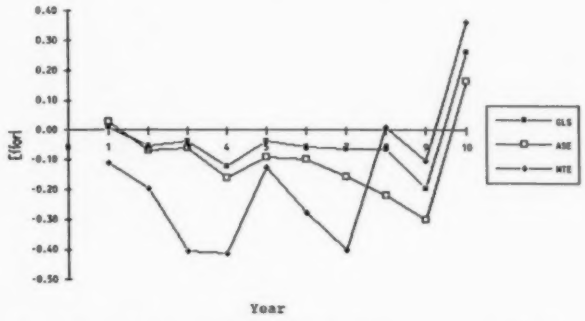



FIG. 4. Error in the estimated efforts for four age groups using three different methods. GLS refers to generalized least squares method, ASE to average of estimates done separately for each age pair, and MTE is based on the overall mortality rate for age groups (marginal totals).

We begin the iterative procedure by choosing reasonable initial values for $s_1$ and $s_2$ and by calculating $\bar{E}$ from the last equation in Eq. 9 which reduces to $\bar{z}_{34} = q\bar{E} + M$ when $s_3 = s_4 = 1$. Next, we calculate

$$(10)\quad \Delta\bar{z}_{12} = \bar{z}_{12} - \left[\ln s_1 - \ln s_2 + \tfrac{1}{2} q\,(s_1 + s_2)\,\bar{E} + M\right]$$

$$\Delta\bar{z}_{23} = \bar{z}_{23} - \left[\ln s_2 + \tfrac{1}{2} q\,(s_2 + 1)\,\bar{E} + M\right].$$

We now look for corrections on the initial estimates of $s_1$ and $s_2$ that make the estimated $\Delta\bar{z}_{12}$ and $\Delta\bar{z}_{23}$ zero. To that end, we replace $s_1$ and $s_2$ with $s_1(1 + \Delta s_1)$ and $s_2(1 + \Delta s_2)$ and make use of the approximation $\ln(1 + \Delta s_i) = \Delta s_i$ to linearize the contributions of $\Delta s_i$'s in Eq. 10. The result, written in matrix format, is

$$\begin{pmatrix} \Delta\bar{z}_{12} \\ \Delta\bar{z}_{23} \end{pmatrix} = \begin{pmatrix} a_1 & b_1 \\ 0 & c_2 \end{pmatrix} \begin{pmatrix} \Delta s_1 \\ \Delta s_2 \end{pmatrix}$$

where $a_1 = \frac{1}{2} q s_1 \bar{E} + 1$, $b_2 = \frac{1}{2} q s_2 \bar{E} - 1$, and $c_2 = \frac{1}{2} q s_2 \bar{E} + 1$. We now solve for $\Delta s_1$ and $\Delta s_2$. Using $s_1(1 + \Delta s_1)$ and $s_2(1 + \Delta s_2)$ as our next estimates of selectivities, we recalculate $\Delta\bar{z}_{i,i+1}$'s as before and then $\Delta s_i$'s again, and so on. The iterative procedure converges fairly rapidly.

The iterative procedure was tried on six simulated data sets. We added an error component on the catchabilities and on the catches as in Table 1. The age–specific catchabilities were set at 0.5 and 0.75. The results based on 100 runs of each combination are shown in Table 3.

For each data set the estimated values of $s_1$ and $s_2$ were close to the true values (0.5 and 0.75) with standard errors of the estimates ranging between 0.039 and 0.054 for $s_1$ and between 0.044 and 0.064 for $s_2$ when error either in catches or in catches and selectivities was set around 20%. The results are independent of the initial values of $s_1$ and $s_2$.

## Application

The method was applied to data on the lake Whitefish (*Coregonus clupeaformis*) fishery in eastern Lake Ontario from 1953 to 1971. The data were obtained from J. Christie and J. Casselman, Glenora Fisheries Station, Picton, Ont.

The fishery is a gillnet fishery and the likelihood of capture depends on the size of the fish. Based on existing gillnet selectivity data and observed size-at-age frequencies, the selectivities of three major age groups (ages 4, 5, and 6) were calculated for each year. As there were substantial differences in growth from year to year, the selectivities changed both with age and year. The separability condition was not obviously met. However, since the selectivities are known, we can still estimate the effective effort. Eq. 7 is now written as

$$(7')\quad \Delta\mathbf{z} = \begin{pmatrix} A_1 \\ A_2 \\ (1)' \end{pmatrix} \Delta\mathbf{E} = \mathbf{B}\,\Delta\mathbf{E}.$$

Both $\mathbf{A}_1$ and $\mathbf{A}_2$ have the same structure as $\mathbf{A}$ in Eq. 6. The individual elements are calculated slightly differently. The elements of $\mathbf{A}_1$, for $q = 1$, are given as

$$a_i = 1 + \frac{1}{2} s_{1j} E_{kj}$$

$$b_i = -1 + \frac{1}{2} s_{2j+1} E_{kj+1}$$

and those in $\mathbf{A}_2$ as above, except that $s_{1,j}$, $s_{2j+1}$ are replaced with $s_{2j}$, $s_{3j+1}$.

For this fishery, we also have data on total effort. Two measures of effort are available. One is based on total yards of net fished each year and the other is "corrected" yardage, corrected for assumed gear saturation in years of high fishing effort.

We have estimated the annual effective efforts, calculated by iteration from Eq. 7′. Interestingly, our effective effort (= estimated fishing mortality) correlates better with the "corrected" yardage than with the total. In Fig. 5, we have plotted our effective effort against the total corrected yardage.

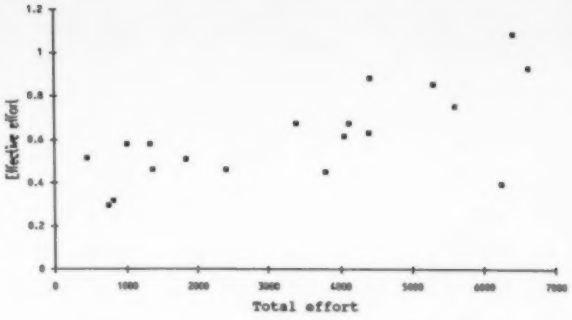



FIG. 5. Relationship between the estimated and total (= corrected yardage) effort in the Lake Ontario lake whitefish fishery.

## Discussion

We have given a simple algorithm to estimate effective effort from catch-at-age data. The algorithm presupposes that the average log decline in catch-at-age is an estimate of the true average mortality rate, corrected if necessary for differences in selectivities at age. It turns out that this log decline is a biased estimate of the average mortality rate, the bias being $\frac{1}{n}(\ln E_1 - \ln E_{n+1})$ where $n$ is the number of years minus 1 and $E_1$ and $E_{n+1}$ are the efforts in the first and last year. Alternatively, we could have done as Pope and Shepherd (1982) did and arbitrarily fixed the effective effort (or the fishing mortality) at the final year and then solved Eq. 5 (with appropriate modification) iteratively. While our feeling is that equating the log decline of catch-at-age with the average mortality is not nearly so arbitrary, there may be little to chose between the two methods if data are available for two ages only. However, with three or more age groups, our method has been extended to a generalized least squares procedure that permits inclusion of an appropriate error structure.

The method was verified using simulated data with a specified error structure. We generated catches-at-age from randomly distributed population sizes at the age of entry to the fishery using randomly distributed effective efforts. The age-specific selectivities were allowed to fluctuate with a 20% error limit. In addition, we let the observed catches differ from the actual catches by up to 20%.

The equations used for determining effective effort do not have a unique solution. Moreover the estimated values depend on an assumed natural mortality rate which in the examples considered has been at times 60% above or 40% below the actual or true value. Nevertheless, and in spite of all this, our estimates of the effective effort correlate well with the actual effective efforts used in generating the basic data. We therefore believe that in the majority of cases the proposed algorithm does recover, if not the actual effective efforts, at least estimated efforts that are closely correlated with true fishing mortalities.

When the true $M$ is higher than the assumed $M$ ($Me$) the effective efforts or fishing mortalities are overestimated and conversely, when $M < Me$, they are underestimated. This is expected because the algorithm partitions the total decline in catch-at-age from one year to the next to its components.

Similarly, changes in selectivities can also be "absorbed" into $M$ because the constant term in Eq. 2 is $M + \ln s_i - \ln s_{i+1}$. In theory, if $M$ is in fact constant (and known) over all ages in the database the selectivities can be estimated as shown. However, their values are dependent on how good our guess of $M$ is. Too high a value of $Me$ will tend to overestimate the differences between $s_i$'s, and conversely, a low value of $Me$ will tend to even out the real differences. Moreover, if $M$ is not constant over age, then any changes in true $M$ will be reflected in our estimates of the selectivities.

A good indication of whether $M$ and/or selectivities are changing with age is obtained by comparing the annual effective efforts estimated separately for each age pair. If, for instance, the estimated effective efforts increase with age, this may be caused by either a change in $M$ with age or a change in the "selectivities." It will be difficult to distinguish between changes in $M$ and in the selectivities without some additional information.

We applied the algorithm to estimate the effective efforts (= fishing mortalities) in the lake whitefish fishery in eastern Lake Ontario. When the estimates were compared with the "field" data, we observed a nonlinear relationship between the estimated fishing mortality and the observed effort, akin to the findings of Crecco and Overholz (1990). It appears that in this fishery, low levels of effort generate proportionally larger fishing mortality rates than higher levels. However, the low effort levels are usually associated with low fish abundance. It may well be that when the abundance is low, fish are more concentrated (occupy smaller area) than in years of high abundance and hence are easier to catch, relatively speaking.

## Acknowledgments

This research was funded by an NSERC grant to J.E. Paloheimo and an NSERC postgraduate scholarship to Y. Chen. We also thank John Casselman of Glenora Fisheries Station, Picton, Ont., for access to lake whitefish data and Jack Christie, also from Glenora, for support and help in the data analysis.

## References

CRECCO, V., AND W.J. OVERHOLZ. 1990. The causes of density-dependent catchability for Georges Bank haddock *Melanogrammus aeglefinus*. Can. J. Fish. Aquat. Sci. 47: 385–394.

DERISO, R.B., T.J. QUINN II, AND P.R. NEAL. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. 42: 815–824.

DOUBLEDAY, W.G. 1976. A least squares approach to analyzing catch-at-age data. Res. Bull. Int. Comm. Northwest Atl. Fish. 12: 69–81.

FOURNIER, D., AND C.P. ARCHIBALD. 1982. A general theory for analyzing catch-at-age data. Can. J. Fish. Aquat. Sci. 39: 1195–1207.

PALOHEIMO, J.E. 1961. Studies on estimation of mortalities: 1: comparison of a method described by Beverton and Holt and a new linear formula. J. Fish. Res. Board Can. 18: 645–652.

PALOHEIMO, J.E. 1980. Estimation of mortality rates in fish populations. Trans. Am. Fish. Soc. 109: 378–386.

PALOHEIMO, J.E., AND L.M. DICKIE. 1964. Abundance and fishing success. Cons. Int. Explor. Mer Rapp. P.-V. 152–163.

POPE, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Res. Bull. Int. Comm. Northwest Atl. Fish. 9: 65–74.

POPE, J.G., AND J.G. SHEPHERD. 1982. A simple method for the consistent interpretation of catch-at-age data. J. Cons. Int. Explor. Mer 40: 176–184.

SHEPHERD, J.G., AND M.D. NICHOLSON. 1991. Multiplicative modeling of catch-at-age data, and its application to catch forecasts. J. Cons. Int. Explor. Mer 47: 284–294.

## Appendix

We write Eq. 2 in a somewhat more general form by including the possible changes in annual age-specific catchabilities:

$$(2'') \quad z_{i,j+1,j} = \ln E_j - \ln E_{j+1} - \ln s_{ij} - \ln s_{i+1,j+1} + \frac{q}{2}(s_{ij} E_j + s_{i+1,j+1} E_{j+1}) + M .$$

From the above, we can see that the errors in $\Delta z_{i,j+1,j}$, call them $e_{ij}$, can be written approximately as

$$e_{ij} = \Delta C_{ij} - \Delta C_{i+1,j+1} - (1 + \tfrac{1}{2} q E_j)\,\Delta s_{ij} + (-1 + \tfrac{1}{2} q E_{j+1})\,\Delta s_{i+1,j+1} .$$

Denoting the variances of catches at age $i$ by $\sigma_{cci}$ and the variance of catchabilities by $\sigma_{ss}$, it follows from above that

$$\text{Var}(e_{ij}) = \sigma_{cci} + \sigma_{cci+1} + (1 + \tfrac{1}{2} q E_j)^2 \sigma_{ss} + (-1 + \tfrac{1}{2} q E_{j+1})^2 \sigma_{ss}$$

$$\text{Cov}(e_{ij}, e_{i+1,j+1}) = (\tfrac{1}{4} q^2 E_{j+1}^2 - 1)\,\sigma_{ss} - \sigma_{cci+1}$$

$$\text{Cov}(e_{ij}, e_{i'j'}) = 0 \text{ whenever } i - i' > 1, j - j' > 1 .$$

Note that the value of **V**, the elements of which were given above, depends on the parameters to be estimated ($E_j$'s) as well as on the unknown values of the errors in catches and catchabilities. However, the estimates are not very sensitive to the actual values of the elements in **V** as long as the pattern is more or less correct in the sense that the elements on the diagonal are positive and those on the subdiagonals are negative. In the simulations that we ran the **V** matrix was not updated to account for changes in $E_i$'s. Moreover, $\sigma_{cci}$'s were also taken as constant.

To accommodate the inevitable increase in variance with age in real data, we suggest that $\sigma_{cci}$'s be made proportional to the logarithm of the percent marginal catches-at-age.

The **V** matrix is a $3n + 1 \times 3n + 1$ matrix. The variances and covariances defined above give us the first $3n$ rows and columns. The last row and column are zeros on the account of the condition $\Sigma \Delta E_j = 0$. To avoid the numerical somersaults needed to get around the singular **V** matrix, we have replaced the last zero on the principal diagonal by a small number (e.g., 0.001). This means that the condition $\Sigma \Delta E_j = 0$ is given a substantial weight but not made into an absolute condition.

# Pup Production of Harp Seals, *Phoca groenlandica*, in the Northwest Atlantic

G.B. Stenson and R.A. Myers

*Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada*

M.O. Hammill

*Department of Fisheries and Oceans, Science Branch, P.O. Box 1000, Mont Joli, QC G5H 3Z4, Canada*

I-H. Ni

*Department of Biology, The Hong Kong University of Science and Technology, 5/F World Shipping Centre, 7 Canton Road, Tsimshatsui, Kowloon, Hong Kong*

W.G. Warren

*Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada*

and M.C.S. Kingsley

*Department of Fisheries and Oceans, Science Branch, P.O. Box 1000, Mont Joli, QC G5H 3Z4, Canada*

Stenson, G.B., R.A. Myers, M.O. Hammill, I-H. Ni, W.G. Warren, and M.C.S. Kingsley. 1993. Pup production of harp seals, *Phoca groenlandica*, in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 50: 2429–2439.

Northwest Atlantic harp seal, *Phoca groenlandica*, pup production was estimated from aerial surveys flown off eastern Newfoundland ("Front") and in the Gulf of St. Lawrence ("Gulf") during March 1990. One visual and two independent photographic estimates were obtained at the Front; a single photographic estimate was obtained in the Gulf. Photographic estimates were corrected for misidentified pups by comparing black-and-white photographs with ultraviolet imagery. Estimates were also corrected for pups absent from the ice at the time of the survey using distinct age-related developmental stages. Stage durations in the Gulf appeared consistent with previous studies but were increased by 30% to improve the fit to staging data collected at the Front. The best estimate of pup production at the Front was obtained from the visual surveys. A total of 467 000 (SE = 31 000) pups were born in three whelping concentrations. The photographic estimates were comparable. Pup production estimates for the southern (Magdalen Island) and northern (Mecatina) Gulf whelping patches were 106 000 (SE = 23 000) and 4 400 (SE = 1300), respectively. Thus, total pup production was estimated to be 578 000 (SE = 39 000).

Le nombre de naissances de phoque du Groenland, *Phoca groenlandica*, dans l'Atlantique nord-ouest a été estimé par des relevés aériens effectués dans l'est de Terre-Neuve (région du «Front») et dans le golfe du Saint-Laurent (région du «Golfe») en mars 1990. On a obtenu une estimation visuelle et deux estimations photographiques indépendantes pour la région du Front et une seule estimation photographique pour la région du Golfe. On a ajusté les estimations photographiques afin de tenir compte des rejetons mal identifiés en comparant des photographies en noir et blanc à des images prises à ultra-violet. Les estimations ont également été corrigées au moyen des stades de développement distincts liés à l'âge pour tenir compte des rejetons qui n'étaient pas sur la banquise au moment du relevé. La durée des stades de développement dans le Golfe semblait correspondre aux données d'études antérieures, mais elle a été augmentée de 30 % pour mieux correspondre aux données existantes dans la région du Front. La meilleure estimation des naissances dans la région du Front a été obtenue par les relevés visuels. Un total de 467 000 (erreur-type = 31 000) rejetons sont nés dans les trois aires de mise bas. Les estimations photographiques étaient comparables. Les estimations du nombre de naissances pour les aires de mise bas dans la partie sud du Golfe (îles de la Madeleine) et dans la partie nord (Mecatina) étaient respectivement de 106 000 (erreur-type = 23 000) et 4 400 (erreur-type = 1 300). On estime donc à 578 000 (erreur-type = 39 000) le nombre total de naissances.

*Received January 12, 1993*
*Accepted May 31, 1993*
*(JB754)*

*Reçu le 12 janvier 1993*
*Accepté le 31 mai 1993*

The harp seal, *Phoca groenlandica*, is an abundant, ice-breeding phocid which migrates annually between the sub-Arctic and Arctic regions of the Atlantic. Harp seals are separated into three populations, northwest Atlantic, White Sea, and Greenland Sea (Jan Mayen), based on their whelping locations. The northwest Atlantic population, the largest of the three, divides into two herds: one which whelps off the coast of northern Newfoundland or southern Labrador ("Front") and the other in the Gulf of St. Lawrence ("Gulf").

As the most abundant seal along the Atlantic coast of Canada, the harp seal has been subjected to a large commercial hunt since the early eighteenth century. Between 1950 and early 1970s

when harvest quotas were introduced, this hunt may have reduced harp seal pup production in the northwest Atlantic by an estimated 50% of the approximately 600 000 pups born in the early 1950s (Lett and Benjaminsen 1977; Winters 1978). Prior to 1983, the large-vessel take of pups on the whelping patch accounted for the majority of the hunt-related mortality. A ban on the importation of young (whitecoat) pelts implemented by the European Economic Community in 1983 destroyed the market for seal pelts, ending the traditional large-vessel hunt.

Harp seals are still hunted by land-based sealers in both the Gulf and Front areas from November through May and in Arctic waters during the summer. However, the catch declined from an annual average of 172 000 between 1978 and 1982 to 49 400 between 1983 and 1990 (Anonymous 1991). This decline has lead to the perception that the population has increased and that it may be associated with the decline in commercial fish species in the area (Anonymous 1986; Harris 1990). Although considerable effort was expended to obtain information on the dynamics and size of this population in the 1970s and early 1980s (Sergeant 1971; Allen 1975; Benjaminsen and Øritsland 1975; Lavigne et al. 1979, 1980; Bowen and Sergeant 1983; Roff and Bowen 1983, 1986), the last estimate of population size was based on data obtained in 1983 (Bowen and Sergeant 1985; Myers and Bowen 1989) and does not reflect the decline in hunt-related mortality.

This paper presents estimates of northwest Atlantic harp seal pup production in 1990 using a combination of systematic visual and photographic aerial surveys, corrected for the temporal distribution of births. It also presents a technique to correct errors in reading photographs by comparing images obtained from separate wavelengths.

## Materials and Methods

### Reconnaissance Survey

Whelping concentrations were located using fixed-wing and helicopter reconnaissance surveys of areas historically used by harp seals. At the Front and in the northern Gulf of St. Lawrence, fixed-wing reconnaissance flights were conducted during 5–25 March (Fig. 1). Repeated systematic east–west transects, spaced 18.5 km apart, were flown at an altitude of 230 m from the inner edge of the ice pack to the outer edge between 50° and 54°42′N at the Front. The ice was unsuitable for seals south of the survey area. Three whelping concentrations (Main, South, and Belle Isle) were located (Fig. 2). Reconnaissance flights between 50° and 51°40′N in the northern Gulf located a group of whelping seals (Mecatina patch) at 50°09′N, 58°55′W. In the southern Gulf, concentrations of whelping seals were located approximately 70 km north–northwest of the Magdalen Islands on 22 February. During the survey period, these groups coalesced and drifted eastward passing to the north of the Islands. No additional concentrations were located during additional reconnaissance flights conducted until late March.

### Photographic Surveys

Fixed-wing aerial photographic surveys were flown using a 23 × 23 cm format metric mapping camera (Zeiss RMK/A) equipped with a 150-mm Sonnar lens and Kodak Double-X (2405) aerographic black-and-white film. All surveys were flown at an altitude of 305 m providing coverage of a 455.5 × 455.5 m area per photo. Correct altitude and transect spacing was maintained using a radar altimeter and Loran C. There was no overlap between consecutive frames.

Images were also obtained using a Vinten 70-mm aerial reconnaissance camera fitted with a 76.2-mm quartz lens, a Wratten 18A ultraviolet filter, and Kodak Tri-X (2403) black-and-white aerographic film. This system obtained images in the ultraviolet wavelengths (300–400 nm) which increase the visibility of white-coated pups (Lavigne 1976). Each photograph covered an area of ice 228.6 × 228.6 m.

Two strata were identified at the Front: whelping concentrations (high density) and scattered pups outside whelping patches (low density). Systematic surveys of three whelping concentrations were carried out on 15–19 March. Each patch was surveyed once and, with the exception of the Main patch, each survey was completed within a single day. To estimate the number of pups born outside the whelping patches, a series of systematic east–west transects, 37 km apart, were flown between 54° and 50°20′N between 13 and 28 March (Fig. 2). Photographs began when the ice was considered to be heavy enough to support seals and terminated when the ice was considered to be too thin.

In the Gulf, a systematic survey of the southern (Magdalen Islands) whelping concentration was flown on 8 March. Animals in the northern Gulf (Mecatina patch) were surveyed 14 March.

All surveys were based on a systematic sampling design with a single random start. The sampling unit was a transect of variable length. The estimate of the number of pups for each survey region was the sum of pups on the photographs over all transects, correcting for noncontiguous photographs and the transect spacing. The estimates of error variance were based on serial difference between transects (Cochran 1977, p. 225; Kingsley et al. 1985). Because photographs did not overlap, it was assumed that the distribution and density of pups on the unobserved portions were similar to those in the observed portions. The additional component of error that arises from this assumption was judged to be small and, to some extent, is included in the between-transect variability.

Groups of adjacent transects were defined based on homogeneous transect spacing. For each group, a weighting factor $k_i$ was calculated as

$$(1)\quad k_i = S_i/W_i$$

where $S_i$ = transect spacing (kilometres) for the $i$th group and $W_i$ = transect width (kilometres) for the $i$th group.

The number of pups present were summed over transects ($x_j$). For photographic surveys where frames did not overlap:

$$(2)\quad x_j = \frac{\sum_{z=1}^{f_j} t_{jz} \times l_j}{f_j \times p_j}$$

where $f_j$ = the number of photographs on transect line $j$, $t_{jz}$ = the number of seals in the $z$th frame on the $j$th transect, $l_j$ = the total transect length, and $p_j$ = the frame length.

The estimated numbers of pups for the $i$th survey is given by

$$(3)\quad \hat{N}_i = k_i \sum_{j=1}^{J_i} x_j$$

where $J_i$ = the number of transects in the $i$th survey; variance was calculated as

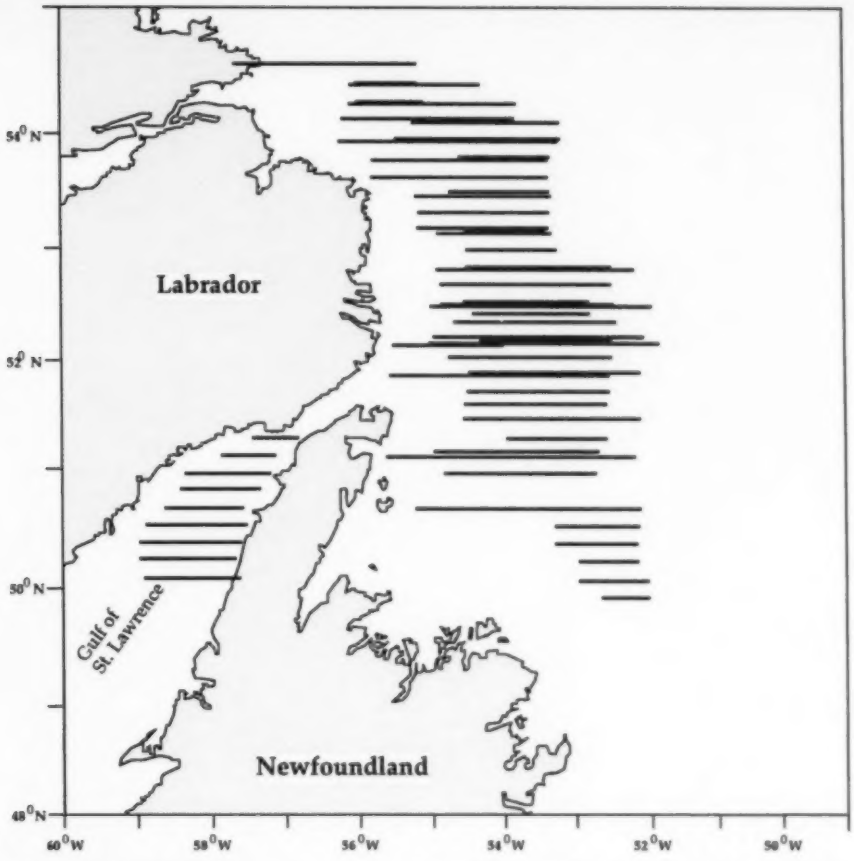



FIG. 1. Aerial reconnaissance surveys conducted at the Front and the northestern Gulf of St. Lawrence, 5–28 March 1990.

$$(4)\quad V_i = \frac{k_i \times (k_i - 1) \times J_i}{2(J_i - 1)} \sum_{j=1}^{J_i - 1} (x_j - x_{j+1})^2.$$

If transect spacing changed within the survey area, each area of homogeneous transect spacing was treated as a separate survey with the estimated number of pups given by

$$(5)\quad \hat{N}_i = k_i \left[ x_{i1} / 2 + \sum_{j=2}^{J_i - 1} x_{ij} + x_{iJ_i} / 2 \right]$$

where $J_i$ = the number of transects in the $i$th group, $x_{ij}$ = the number of pups counted on the $j$th transect in the $i$th group, and the end transects are the limits of the survey area.

Rewriting the variance estimate given in Equation 4 results in

$$(6)\quad V_i = \frac{k_i \times (k_i - 1)}{2} \sum_{j=1}^{J_i - 1} (x_j - x_{j+1})^2.$$

The combined estimate for total population and its error variance for the entire population are obtained by

$$(7)\quad \hat{N} = \sum_{i=1}^{I} N_i$$

$$(8)\quad \hat{V} = \sum_{i=1}^{I} V_i$$

where $I$ is the number of groups of transects.

Positive prints from the Front survey were examined by two readers; one read the low-density transects (scattered) and the other read photographs from the whelping concentrations. Each frame was examined using an illuminated hand-lens (7–8× magnification) and the number and position of all seals were recorded on a clear acetate overlay. Before beginning, selected frames were examined by both readers and identified seals compared. This was continued until the cues used to identify seals were similar. The first, experienced, reader read all photographs once and then reread the photographs in the same order until the counts were consistent with original counts. The second, less experienced, reader read all prints twice.

Negative images from the Gulf were examined by a single experienced reader using a dissecting microscope mounted on a light table. Each frame was examined twice. Acetate overlays

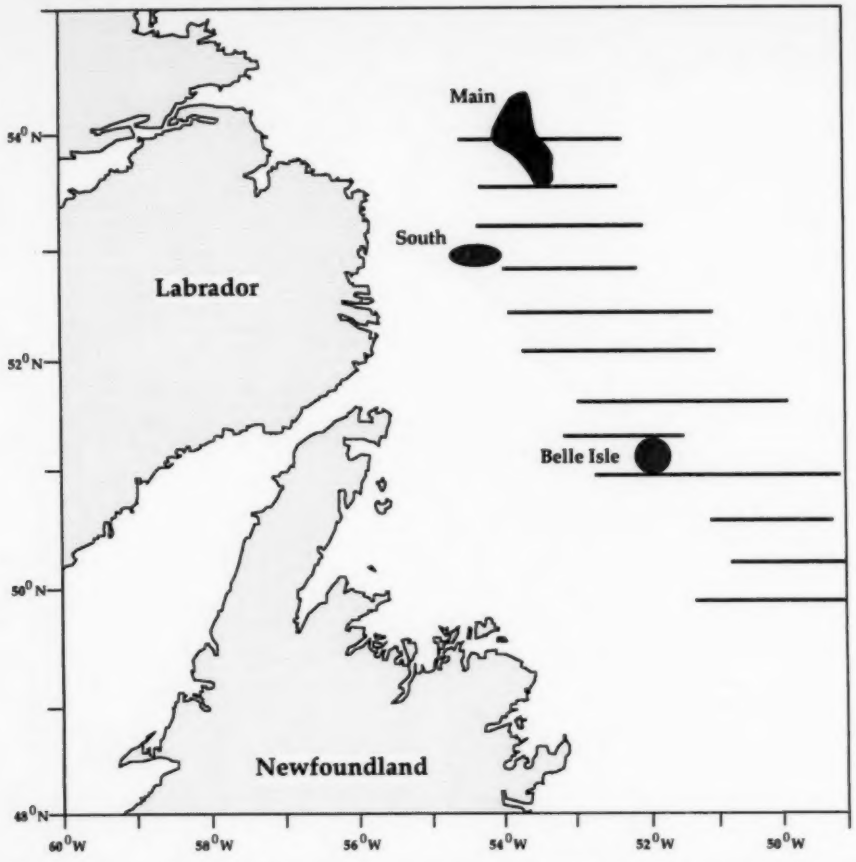



FIG. 2. Positions of low-density photographic transects flown on 13, 19, 20, and 25 March (as estimated on 25 March 1990) providing complete coverage of the survey area. Stipled areas indicate the position of the three whelping concentrations on the day of visual surveys.

TABLE 1. Regression equations developed to correct for missed or incorrectly identified pups on photographs from the Gulf and Front surveys (SE in parentheses).

| | $N$ | Intercept | Slope | $r^2$ |
|---|---|---|---|---|
| Gulf | 296 | 0.1481 (0.223) | 1.495 (0.048) | 0.77 |
| Front | | | | |
| Scattered | 1110 | −0.063 (0.253) | 1.06 (0.178) | 0.76 |
| Concentrations | 391 | −0.097 (0.104) | 0.678 (0.109) | 0.91 |

from the two independent readings were compared to confirm the identified seals.

Film counts were corrected by comparing identical areas on a sample of matched black-and-white (Zeiss) and ultraviolet (Vinten) images. The seals on both films were identified independently by the most experienced reader. Then, individuals on each image were compared to determine if any pups were missed or misidentified. The final, or corrected, count was considered to be the best estimate of actual number of seals present. Zeiss images from this sample were examined by all readers. The corrected counts ($x$) were regressed on the counts for each reader ($y$), $x = a + by$, to develop a correction factor for pups missed during photograph readings. Individual photograph counts were corrected using the appropriate regression for each reader (Table 1). The regressions were not constrained to pass through the origin, and negative estimates of true counts were replaced by zero.

The estimated corrected transect totals are subject to error due to the variation about the regression (measurement error). Bootstrap estimates were obtained for the variance of each estimated transect total. Two hundred bootstrap samples were generated for each of the Front transects and 400 for the Gulf transects, which should be adequate for estimating standard errors (Efron 1981). As a check, the means of the bootstrap samples were also computed. These differed little from the bootstrap estimates. For each collection of transect lines the measurement-error variances of the transects were added to form the total measurement-error for the patch. This in turn was added to the sampling variance.

### Visual Surveys

At the Front, systematic visual surveys of the three whelping concentrations were flown using either one or two ship-based helicopters at an altitude of 46 m. Observers, seated in the left and right rear seats, counted all pups within a 30- or 35-m-wide strip on each side of the aircraft. Each transect began when a third observer, seated in the front, encountered seals and was terminated approximately 5 km after the last seal was observed. The survey ended when no seals were seen on transect and were not observed outside the survey area. The analysis was the same as used for the photographic surveys, assuming complete coverage along a transect.

### Correcting for the Temporal Distribution of Births

Throughout the survey period, pups were classified into seven distinct age-dependent stages based on pelage and morphometric features as described by Stewart and Lavigne (1980). Prior to the surveys, classifications by personnel from both the Front and Gulf areas were standardized to ensure consistency. At the Front, 10–15 random points were chosen along transverse flight lines flown across the long axis of the patch. At each point, two groups, walking in opposite directions, classified all pups encountered within 10 min. In the Gulf, staging locations were randomly chosen. At each location, two observers classified all pups within 10 m of line for a distance of approximately 100 m. In both areas, an entire patch was covered in a single day.

Given information on the proportion of pups in each of these developmental stages at several times and the duration of each stage, it is possible to estimate the distribution of births over the pupping season. This information can then be used to correct an aerial survey estimate of abundance for those pups that have yet to be born at the time of the survey (Bowen et al. 1987; Myers and Bowen 1989). A summary of the methods used to model the stage transitions is given below; see Myers and Bowen (1989) for details.

Developmental stages are denoted by the subscript $j$, and a pup passes from stage $j$ to $j+1$. We specify stage duration in terms of instantaneous transition intensity functions: $\phi_j(\tau) = \lim_{\Delta\tau \to 0}$ (probability an animal passes from stage $j$ to $j+1$ in the interval $[\tau, \tau + \Delta\tau]$)/$\Delta\tau$) where $\tau$ is the time spent in stage $j$. This specifies the force of transition into stage $j+1$ from stage $j$, given that the animal has spent time $\tau$ in stage $j$. Note that the transition intensities depend only on the current stage and the time so far spent in that stage. The rate at which pups enter stage $j$ at time $t$ is denoted by $m_j(t)$ and is given by the recurrence relationship

$$(9) \quad m_j(t) = \int_0^\infty m_{j-1}(t-\tau)\, \phi_{j-1}(\tau)\, d\tau.$$

If there is no mortality, the total number of pups in stage $j$ that can be observed at time $t$, $n_j(t)$, is the integral of the rate pups entered stage $j$ time $\tau$ ago times the probability that those pups have not entered stage $j+1$:

$$(10) \quad n_j(t) = \int_0^\infty m_j(t-\tau) \left(1 - \int_0^\tau \phi_j(s)\, ds\right) d\tau.$$

Equations 9 and 10 adequately describe stages 1–5. One minor modification of the model is needed. By stage 6, a substantial fraction of pups have left the ice and entered the water. Because these cannot be seen in the aerial photographs, we also estimate the fraction of stage 6 pups that are not visible.

TABLE 2. Uncorrected photographic and visual estimates of pup production (thousands) at the Front and in the Gulf of St. Lawrence during March 1990 (SE in parentheses). Low-density photographic transects at the Front resulted in an estimate of 536 400 (SE = 115 300).

| Patch | Date | Visual | Date | Photographic |
|---|---|---|---|---|
| Front | | | | |
| Main | 12 | 293.3 (19.5) | 15–16 | 370.3 (45.2) |
| South | 16 | 114.7 (17.0) | 16 | 114.2 (83.8) |
| Belle Isle | 19 | 7.8 (1.4) | 19 | 6.2 (1.4) |
| Gulf | | | | |
| Magdalen | | | 8 | 103.1 (22.9) |
| Mecatina | | | 14 | 4.4 (1.3) |

The transition intensity $\phi_j$, of stages 1, 2, 3, and 4 was assumed to follow a gamma density. That is, $\phi_j(t) = \rho_j(\rho_j t)^{\kappa_j - 1} e^{-\rho_j t} / \Gamma(\kappa_j)$ where $\kappa_j$ is the shape parameter, $\rho_j$ is the scale parameter, and $\Gamma(\,)$ is the gamma function. These parameters were estimated in Myers and Bowen (1989).

Four parameters need to be estimated to describe the model: three to specify the location and shape of the probability distribution of births over time, $m_1(t)$, and one describes the fraction of stage 6 pups that are visible. They are estimated using a maximum likelihood method described in Myers and Bowen (1989). The model output provides an estimate of the fraction of the total pups born that are visible to be photographed on any day.

The confidence limits for the parameters of the birthing distribution were calculated by plotting the log-likelihood for pairs of parameter values. The approximate 95% confidence region was chosen as the set of parameter values where the difference in the log-likelihood was greater than 4 from the maximum. The confidence region of the proportion of the total pup production that is visible on the day of the survey was obtained by determining the maximum and minimum proportion of pups visible within the 95% confidence region for the parameters of the birthing distribution.

## Results

### Photographic Surveys

Due to high winds (> 75 km/h) at the Front which caused the patch to spread over an extremely large area, the northern part of the Main patch was surveyed on 15 March, while the southern area was completed on 16 March. The amount of drift which occurred between the two survey days was estimated from the location of a satellite transmitter placed on the ice in the Belle Isle patch which was situated further south (51°10′N, 53°05′W). Fifteen transects were flown. Transect spacing varied, resulting in two strata with a transect interval of 14.8 km separated by a stratum with transects 7.4 km apart. A total of 1.8% of the area was photographed. The resulting pup production estimate, uncorrected for births, was 370 258 (SE = 45 250) (Table 2). This estimate is negatively biased, as at least one small group of pups ($n$ = 30–50), previously marked within the Main patch, was located outside the survey area on 16 March. It is unknown if other groups were missed.

The South patch was also surveyed on 16 March using three transects spaced 7.4 km apart, resulting in 2.4% of the area being photographed. Pup production was estimated to be 114 167

(Table 2). Extreme heterogeneity among transects resulted in a standard error of 83 807. Five transects, spaced 3.7 km apart, were flown over the Belle Isle patch on 19 March (Table 2). Photographic coverage was 4.6% and pup production was estimated to be 6 162 (SE = 1 404).

Photographic transects of the low-density stratum were flown on six separate days between 13 and 28 March. By matching lines and estimating ice drift based on the position of satellite beacons, transects flown on 13, 19, 20, and 25 March were considered to be independent and were combined to give complete coverage of the entire survey area (Fig. 2). These surveys resulted in an estimate of total pup production at the Front of 536 375 (SE = 115 297) (Tables 2 and 5). Because of the high degree of ice movement and mixing of the patches due to high winds, the location of the whelping concentrations could not be identified on the photographs. Therefore, this estimate includes both seals born in the concentrations and those which may have been born outside the patches.

The southern Gulf whelping concentration was surveyed 8 March (Fig. 3). Twenty-six transects were flown with the patch divided into four strata: spacings between the individual transects were 14.8, 7.4, 3.7, and 7.4 km, respectively, resulting in a total photographic coverage of 4.3%. Pup production, uncorrected for the distribution of births, was 103 075 (SE = 22 942) (Table 2). In the northern Gulf, a photographic survey of the Mecatina patch was conducted on 14 March, consisting of six transects flown at 1.85-km intervals (Fig. 3). Photographic coverage was 19%. Pup production was estimated to be 4 373 (SE = 1 264) (Table 2).

### Visual Surveys

All three whelping patches at the Front were visually surveyed. On12 March the Main patch was surveyed using two helicopters flying a total of 50 transects spaced 1.85 km apart (Table 2). The results of one observer were discarded due to difficulties maintaining the correct strip width, resulting in reduced coverage in the northern half. Total survey coverage was 2.3%. Mean density was 385.2 pups/km$^2$ (SE = 31.8, $n$ = 78) and total pup production, uncorrected for the distribution of births, was 293 338 (SE = 19 460) (Table 2).

The South patch was surveyed on 16 March. Three north–south transects spaced 2.2 km apart and seven spaced 3.3 km apart resulted in a total coverage of 1.8%. Thirteen east–west transects, spaced 0.9 km apart, were flown over the Belle Isle patch on 19 March. Survey coverage was estimated to be 7.0%. Mean density of the South and Belle Isle patches was 533.1 pups/km$^2$ (SE = 77.8, $n$ = 22) and 124.2 pups/km$^2$ (SE = 23.2, $n$ = 26), respectively. The uncorrected estimates of pup production were 114 685 (SE = 16 999) for the South patch and 7 793 (SE = 1 438) for the Belle Isle patch (Table 2).

### Correction for Temporal Distribution of Births

Owing to the large distances separating the patches at the Front, developmental stage determinations of pups in the Main (7–10 and 15–16 March) and South (12 and 17 March) patches occured early in the survey period, while those in the Belle Isle patch were conducted later (19 and 26 March) (Table 3). The birthing ogive was determined by fitting the beta, Weibull, and log-logistic distribution functions to the stage data. Based on maximum likelihood, the Weibull function provided the best fit to the data followed by the beta and the log-logistic functions. However, overall, these functions did not describe the distribution of births at the Front very well, suggesting that changes had occurred in the transition rates of pup stages compared with those described by Myers and Bowen (1989). Unfortunately, stage durations were not determined directly in 1990. However, they could be estimated from available data by assuming that the relative durations were the same between stages. A common factor can then be used to multiply all the stage durations. Two approaches were tried. First, this factor was estimated directly from the survey data by adding an additional parameter to the likelihood function to account for a longer stage duration. This improved the fit of the model significantly (likehood ratio test, $p < 0.05$) and was consistent with the data. We obtained the best improvement if we assumed that the Myers and Bowen (1989) stage durations were 70% of those observed in 1990. The second approach estimated this factor based on resightings of a known group of pups. On March 7, a radio transmitter was deployed on an ice pan and the stages of pups on the ice pan recorded. One stage 1, 10 stage 2, and 15 stage 3 pups were counted. The same group of pups was examined again on March 16 when one stage 1, 16 stage 3, and 12 stage 4 pups were recorded. If all stage 3 pups were just 1 d old (the minimum age estimated previously) on March 7 and the stage durations used by Myers and Bowen (1989) are applicable to this population, then 50% of these pups should have been stage 5 by 16 March. However, if we assumed that the Myers and Bowen (1989) stage durations are 70% of those in 1990 and the same relative stage durations were maintained, the expected stage composition of the group on March 16 was very similar to the stages actually recorded.

An estimated 91% (SE = 2.5%) of the pups were present on the ice at the time of the 12 March visual survey of the Main Patch. No correction was applied to the photographic estimates of this patch (15 and 16 March), as 99% of the pups were estimated to be present during this survey. Both visual and photographic surveys of the South patch were conducted on 16 March, when 83% (SE = 3%) of the pups were estimated to be present. No corrections were applied to the Belle Isle patch due to limited data. However, this patch was not surveyed until 19 March and, based on the staging data available, it is likely that most pupping had occurred by this time.

Developmental stage determinations of pups in the southern Gulf were extensive (Table 4). The model fit was good, and 97% of the pups were estimated to have been present during the survey. Staging of pups in the Mecatina patch in the northern Gulf was not carried out.

The estimate of pups born in the whelping concentrations at the Front, corrected for the temporal distribution of births, based on the visual surveys is 467 200 (SE = 31 200) (Table 5). From the photographic surveys of the high-density strata, 514 000 (SE = 110 800) pups were estimated to have been born in the whelping patches. The corrected estimates of pup production in the Gulf were 106 300 (SE = 23 000) in the Magdalen Island patch and 4 373 (SE = 1 264) in the Mecatina patch (Table 5).

## Discussion

This study provides the first estimate of pup production in harp seals since the end of the large vessel hunt in the mid-1980s. Three independent estimates were obtained for production at the Front. However, due to the variety of assumptions associated with estimating ice drift, the knowledge that some pups were missed during the photographic survey of the main patch, and

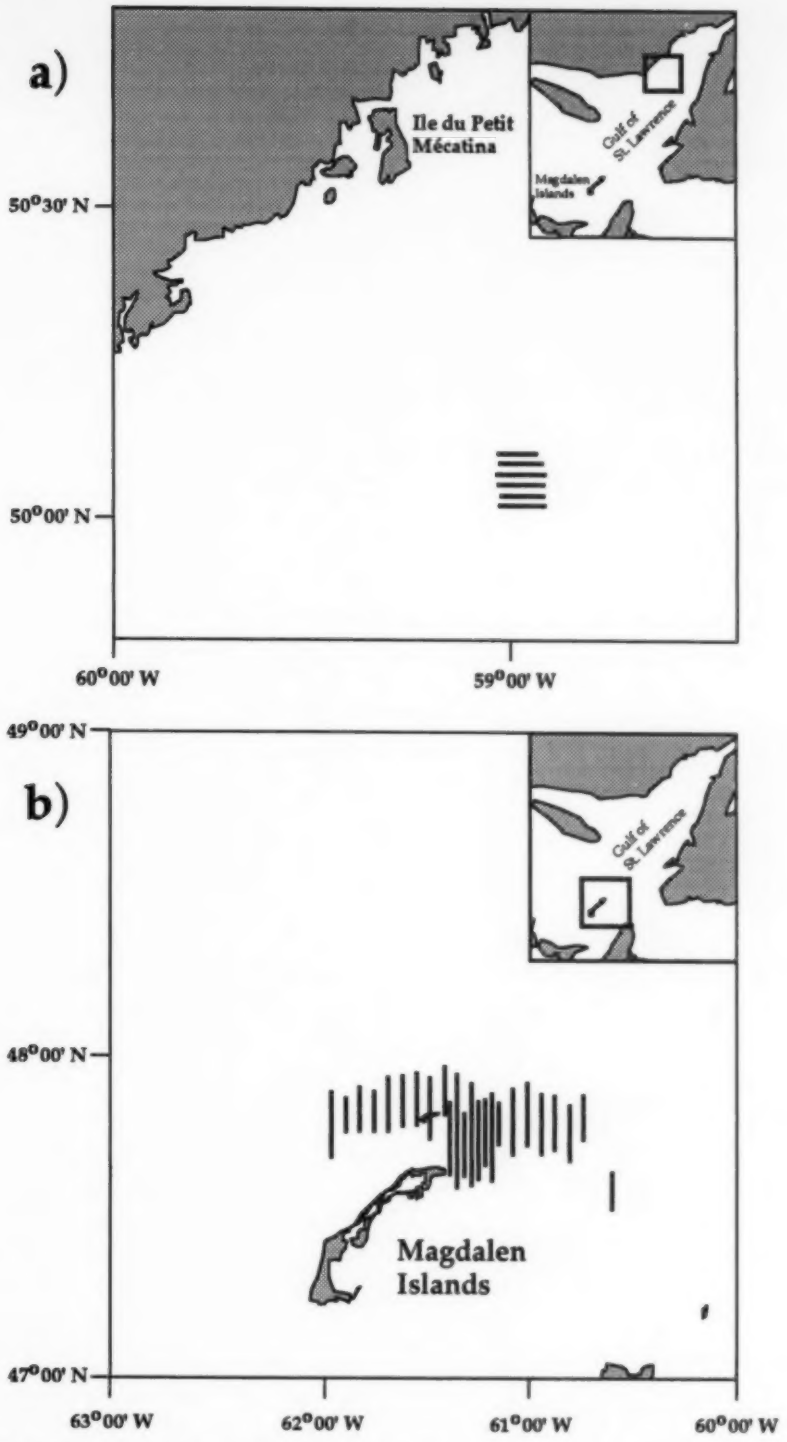



FIG. 3. Photographic surveys conducted in the (a) northern (Mecatina) and (b) southern (Magdalen) Gulf of St. Lawrence during March 1990.

TABLE 3. Numbers of harp seal pups in individual age-dependent stages at the Front during March 1990.

| Date | Patch | Stage 1 | 2 | 3 | 4 | 5 | 6 | 7 | Total |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Main | 20 | 70 | 249 | 0 | 0 | 0 | 0 | 339 |
| 8 | | 0 | 2 | 19 | 0 | 0 | 0 | 0 | 21 |
| 9 | | 1 | 0 | 29 | 0 | 0 | 0 | 0 | 30 |
| 10 | | 23 | 19 | 428 | 0 | 0 | 0 | 0 | 470 |
| 15 | | 1 | 0 | 489 | 99 | 5 | 0 | 0 | 594 |
| 16 | | 1 | 0 | 37 | 77 | 0 | 0 | 0 | 115 |
| 12 | South | 23 | 42 | 67 | 0 | 0 | 0 | 0 | 132 |
| 17 | | 1 | 11 | 605 | 0 | 0 | 0 | 0 | 617 |
| 19 | Belle Isle | 0 | 0 | 388 | 45 | 6 | 0 | 0 | 439 |
| 26 | | 0 | 0 | 0 | 17 | 159 | 8 | 0 | 184 |

TABLE 4. Numbers of harp seal pups in individual age-dependent stages in the southern Gulf during February and March 1990.

| Date | Stage 1 | 2 | 3 | 4 | 5 | 6 | 7 | Total |
|---|---|---|---|---|---|---|---|---|
| Feb. 22 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| 25 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| 26 | 0 | 14 | 71 | 0 | 0 | 0 | 0 | 85 |
| 27 | 0 | 0 | 8 | 1 | 0 | 0 | 0 | 9 |
| Mar. 1 | 10 | 83 | 471 | 54 | 0 | 0 | 0 | 618 |
| 2 | 0 | 22 | 166 | 1 | 0 | 0 | 0 | 189 |
| 4 | 5 | 48 | 407 | 61 | 0 | 0 | 0 | 521 |
| 6 | 0 | 3 | 15 | 10 | 0 | 0 | 0 | 28 |
| 8 | 0 | 9 | 58 | 92 | 0 | 0 | 0 | 159 |
| 10 | 0 | 0 | 18 | 177 | 16 | 0 | 0 | 211 |
| 11 | 0 | 11 | 118 | 318 | 190 | 8 | 0 | 645 |
| 19 | 0 | 0 | 0 | 7 | 15 | 7 | 2 | 31 |

TABLE 5. Photographic and visual estimate of pup production (thousands) at the Front and in the Gulf of St. Lawrence during March 1990 corrected for the temporal distribution of births (SE in parentheses).

| Patch | Visual | Photographs: High density | Photographs: Low density[a] |
|---|---|---|---|
| Front | | | |
| Main | 321.0 (23.0) | 370.3 (45.2) | |
| South | 138.2 (21.1) | 137.6 (101.0) | |
| Belle Isle | 7.8 (1.4) | 6.2 (1.4) | |
| Subtotal | 467.2 (31.2) | 514.0 (110.8) | 536.4 (115.3) |
| Gulf | | | |
| Magdalen | | 106.3 (23.0) | |
| Mecatina | | 4.4 (1.3) | |
| Subtotal | | 110.7 (23.0) | |

[a]Includes pups born outside the whelping concentrations.

large standard error associated with many of the photographic surveys, the visual surveys are considered to provide the best estimates of pup production at the Front. Combining the visual survey estimates for the Front and the photographic surveys in the Gulf results in an estimated total 1990 pup production of 577 900 (SE = 38 800).

Previously, pup production was estimated from age composition data (Sergeant 1971, 1975; Benjaminsen and Øritsland 1975; Winter 1978; Cooke 1985), aerial surveys (Sergeant and Fisher 1960; Sergeant 1971; Lavigne et al. 1980, 1982), and mark–recapture experiments (Bowen and Sergeant 1983, 1985). However, the results were often conflicting, for example, ranging from 251 000 (1975 and 1977, Lavigne et al. 1980, 1982) to 450 000–534 000 (1977–83, Bowen and Sergeant 1983, 1985) for the same period. The Royal Commission on Seals and Sealing in Canada (Anonymous 1986) concluded from available data that pup production in 1978 was probably in the order of 300 000–350 000.

It is difficult to directly compare our results with earlier studies to quantify changes in pup production. Estimates made by different methods are not necessarily comparable, as each technique involves assumptions that may bias the estimates to different degrees. Although aerial survey techniques have been used since 1950 (Sergeant and Fisher 1960; Sergeant 1971; Lavigne et al. 1979, 1980, 1982; Myers and Bowen 1989), difficulties in locating and photographing all of the whelping concentrations resulted in the Gulf and Front not being surveyed satisfactorily in the same year. Unfortunately, it may not be appropriate to combine surveys conducted in different years, since the proportion of pups born in each area may vary among

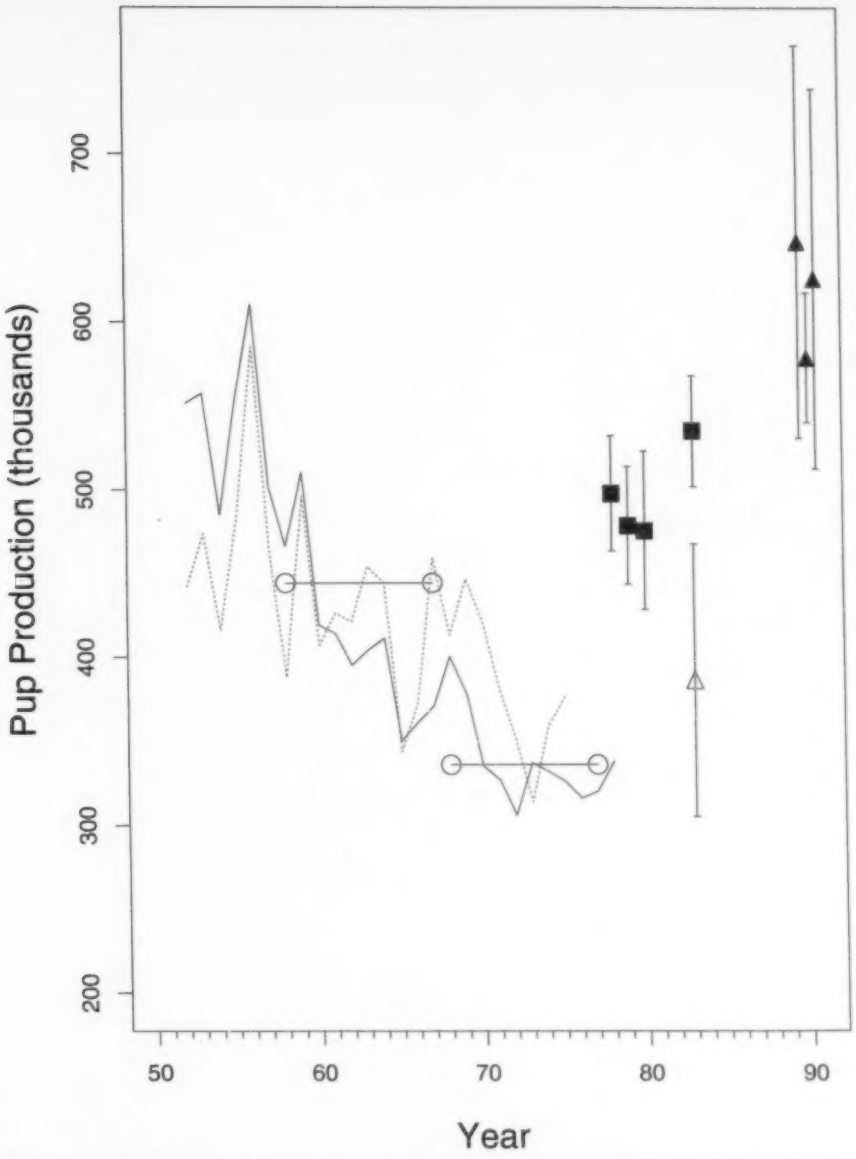



FIG. 4. Pup production estimates of harp seals in the northwest Atlantic: cohort analysis with $M = 0.12$ for 1952–59 and $M = 0.10$ for 1960–78 by Winters (1978) (solid line), cohort analysis with $M = 0.114$ by Lett and Benjaminsen (1977) (broken line), average production for two time periods based on the survival index method by Cooke (1985) assuming moderate aging errors (open circles), mark–recapture estimates by Roff and Bowen (1986) (solid squares ± SE), and aerial survey estimates by Myers and Bowen (1989) (open triangles ± SE) and this paper (solid triangles ± SE).

years (Winters 1978).

The results from a number of studies (Fig. 4) provide an indication of the probable trend in pup production. The two cohort analyses, Lett and Benjaminsen (1977) and Winters (1978), used virtually the same data and yielded similar estimates of the time trend. These estimates are similar to those given by the survival index method, as modified by Cooke (1985), which averaged pup production over a 10-yr period. For comparison, a rough estimate of pup production in 1983 based on an aerial survey at the Front (Myers and Bowen 1989) was included. An independent estimate of pup production in the Gulf and Front obtained from a mark–recapture experiment conducted in the same year (Bowen and Sergeant 1985) indicated that 38% of the total production occurred in the Gulf in that year. Using this ratio and the correcting factors described by Myers and Bowen (1989), an estimate, similar to those obtained from the cohort analysis and survival index methods, was obtained. Comparison of these estimates with our results suggests that an increase in pup production has occurred, possibly to the levels estimated for the 1950s.

Several problems are associated with the use of aerial surveys for harp seals. Their offshore distribution, poor weather

conditions, and unstable ice make it difficult to detect and monitor movements of the whelping patches. Due to the prolonged pupping season (late February through mid-March, Sergeant 1965), not all pups will be present on the ice during any single survey (Myers and Bowen 1989). Finally, the white-coated pup is difficult to detect against the white ice, especially in high-altitude photographs. Therefore, survey results will be biased if scattered individuals or concentrations of whelping animals are missed, if results are not corrected for pups born after the survey is flown or which have left the ice, and if there are errors in counting pups on the photographs (Myers and Bowen 1989).

The largest potential source of error in the estimate of pup production is the failure to detect all whelping concentrations (Myers and Bowen 1989). To minimize this possibility, extensive reconnaissance over historical whelping areas was carried out. It is unlikly that any major patches were missed at the Front. Although the flight lines were spaced 18.5 km apart and, therefore, patches smaller than this could have been missed, multiple flights over the area as the ice drifted southward reduced this possibility.

In the southern Gulf, historical records indicate that a single patch (which may subsequently divide into smaller patches) usually forms to the northwest of the Magdalen Islands. Reconnaissance surveys located only a single patch in 1990. Although no extensive surveys were conducted more than 30 km offshore to the west and southwest of the Magdalen Islands, we believe it unlikely that whelping occurred outside this area, since ice drift would have brought animals closer to the Magdalen Islands before the end of March. No such groups were seen, although reconnaissance around the islands continued until late March.

Harp seals whelp over a period of 3 wk. Myers and Bowen (1989) modelled the distribution of births in 1983 to correct survey estimates for unborn pups, using information obtained from the proportion of pups in distinct age-dependent developmental stages. In their study, peak pupping occurred on 7–8 March, resulting in an optimum survey period for Front harp seals of 10–20 March. With few exceptions, our estimates required little or no correction. Although the visual survey of the Main patch was conducted on 12 March, which was within the optimum period in 1983, only an estimated 91% of the births had occurred in 1990. The staging data suggest that a large pulse of births had occurred around 12 March resulting in a later peak pupping date than in 1983. Peak pupping in the South patch occurred even later. Staging data indicated that only 83% of births had occurred by the time that this patch was surveyed on 16 March. These differences show the variability in timing of births which may occur between patches or years and underline the importance of determining the correction factors necessary for each survey.

The changes observed in the proportion of pups in each developmental stage at the Front are inconsistent with the stage durations used by Myers and Bowen (1989). It is unlikely that these are due to errors in staging because of extensive training and standardization of all observers prior to the surveys. We believe that differences in stage durations may have occurred due to the effects of weather or differences between study sites. Initial development of the transition rates and the model fitting procedure were based on stage durations described by Stewart and Lavigne (1980) for harp seals in the Gulf of St. Lawrence. No inconsistencies were observed when fitting the model to the Gulf stage data, while a significant improvement in the fitting of the birth distributions was obtained if the transition rates for the stages were increased by 30% at the Front. At the Front, harp seals whelp on smaller, less stable pans than animals in the Gulf (G.B. Stenson, personal observation) which may affect attendance patterns in lactating harp seals. Also, a number of storms occurred at the Front in 1990 which may have reduced attendance by the females (Stewart 1987). Since the difference between stage 3 and 4 pups (thin and fat whitecoats) is related to weight gain, the relative duration of these two stages is probably influenced by the nursing rate of pups. If nursing rate or environmental conditions also influence the timing of stage 5, this would account for the differences we observed.

Variability in stage durations will increase the degree of uncertainty associated with the proportion of pups present used to correct the survey estimates. If, in fact, little or no change had occurred in stage durations, then we have overestimated the proportion of pups present and our estimate of total pup production would be too low. Alternatively, if stage durations were longer than the modified durations used, we have underestimated the porportion of the population on the ice and overestimated total pup production. However, correcting for this bias would change our total population estimate by less than 10%. Variability in stage durations should be examined during future surveys, especially at the Front.

Errors in counting seals on film result from difficulties in detecting white pup against a white background, small image size, poor image quality, and photo-reader experience (Myers and Bowen 1989). Lavigne (1976) attempted to improve the visibility of pups by using an ultraviolet (UV) filter, which restricted transmission to the 300–400 nm wavelengths and resulted in a dark pup image against a white background. Studies by Ni et al. (1988) indicated that a quartz lens was required when the UV filter was used. Unfortunately, this combination was only available in a small-format (70-mm) camera, which limited survey coverage. In this study, we flew at 305 m and used both the 70-mm format UV camera and the much larger 23-cm format standard aerographic camera. Comparing images from both systems to correct counts allowed us to take advantage of the larger coverage obtained from the large-format camera while ensuring that pups were not missed due to their low contrast. This exercise also indicated that, at the flying altitude used, many objects originally considered to be pups on the UV images were, in fact, irregularly shaped ice formations or small patches of open water. This may be due to the small image size of a pup (0.2 mm). Therefore, estimates based on UV images alone would overestimate the number of pups present.

A previous attempt was made to correct aerial survey estimates for the number of harp seal pups missed by conventional photography using UV images (Lavigne 1976; Lavigne et al. 1980, 1982). These surveys used pup to adult ratios obtained from low-altitude (300 m), 70-mm-format UV photography to adjust adult counts obtained from complete coverage of the whelping herd using 23-cm-format, high-altitude (1200 m) black-and-white photographs flown at a different time. Unfortunately, this approach does not account for variability in pup to adult ratios due to diurnal or seasonal changes in female attendance (Kovacs 1987; E. Perry and G.B. Stenson, unpublished data) and instead, we used simultaneous black-and-white and UV imagery to obtain an estimate of misidentified pups.

Different correction factors for misidentified pups were

required for each reader, underlining the importance of examining for individual biases among readers. Corrections to counts obtained from the most experienced reader were minimal. Despite considerable training, the counts of the least experienced reader were reduced by 32%, while those of the reader with intermediate experience were increased by 50%. The latter reader identified objects as pups only when flippers could be recognized, while the former was more likely to classify shadows or blowholes as pups than were more experienced readers. We suggest that future surveys should be flown at a much lower altitude, perhaps at 160 m, to increase image size and should employ a motion compensation mechanism to improve image quality. However, this would require an increased number of transects to offset the reduced coverage.

Due to the poor quality of UV images obtained in the Gulf, the correction factor for the Gulf reader was estimated using Front images. The effects of applying correction factors derived from images taken at the Front on the Gulf estimates, if any, are unknown.

In summary, we estimate that 577 900 (SE = 38 800) harp seal pups were born in the northwest Atlantic in 1990, based on an estimated 467 200 pups from the Front, 106 300 in the southern Gulf near the Magdalen Islands, and 4 400 in the Mecatina patch (Table 5).

## Acknowledgements

We thank W. Penney, D. McKinnon, D. Wakeham, W. Hoek, J. Drew, V. Mercer, J. Brattey, E. Perry, and D. Rosen without whose help the surveys would not have been completed. We also thank the aircraft pilots and crew, as well as the crew of the CCGS *Sir John Franklin*, for their invaluable help. We are especially grateful to D. Wakeham, W. Hoek, and E. Perry for reading the photographs and Pat MacDonald of the Department of National Defense for lending us the Vinten camera with quartz lens. Finally, we thank D. Bowen and W. Stobo for assistance in planning the study, J. Lawson for preparing the figures, and P. Shelton, J. Carscadden, and two anonymous referees for reviewing drafts of this paper. Support for this work was provided under the Government of Canada's Atlantic Fisheries Adjustment Program (Northern Cod Science Program).

## References

ALLEN, R.L. 1975. A life table for harp seals in the northwest Atlantic. Rapp. P.-v. Réun. Cons int. Exp. Mer. 169: 303–311

ANONYMOUS. 1986. Seals and sealing in Canada. Report of the Royal Commission. Vol. 3. Supply and Services Canada, Hull, Que.

ANONYMOUS. 1991. Report of the joint ICES/NAFO Working Group on Harp and Hooded Seals. ICES C.M. 1992/Assess: 5: 31 p.

BENJAMINSEN, T., AND T. ØRITSLAND. 1975. The survival of year-classes and estimates of production and sustainable yield of northwest Atlantic harp seals. Int. Comm. Northwest Atl. Fish. Res. Doc. 75/121.

BOWEN, W.D., R.A. MYERS, AND K. HAY. 1987. Abundance estimation of a dispersed, dynamic population: hooded seal (*Cystophora cristata*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 44: 282–295.

BOWEN, W.D., AND D.E. SERGEANT. 1983. Mark–recapture estimates of harp seal pup (*Phoca groenlandica*) production in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 40: 728–742.

BOWEN, W.D., AND D.E. SERGEANT. 1985. A mark–recapture estimate of 1983 harp seal pup production in the northwest Atlantic. NAFO SCR Doc. 85/I/1, Ser. N935: 14 p.

COCHRAN, W.G. 1977. Sampling techniques. 3rd ed. Wiley, New York, N.Y. 428 p.

COOKE, J.G. 1985. Estimation of harp seal (*Phoca groenlandica*) pup production from age samples. Can. J. Fish. Aquat. Sci. 42: 468–473.

EFRON, B. 1981. Non parametric standard errors and confidence intervals. Can. J. Stat. 9: 139–172.

HARRIS, L. 1990. Independent review of the state of the northern cod stock. Final Report of the Northern Cod Review Panel. Department of Fisheries and Oceans, Ottawa, Ont.

KINGSLEY, M.C.S., I. STIRLING, AND W. CALVERT. 1985. The distribution and abundance of seals in the Canadian high Arctic. Can. J. Fish. Aquat. Sci. 42: 1189–1210.

KOVACS, K.M. 1987. Maternal behaviour and early behavioural ontogeny of harp seals, *Phoca groenlandica*. Anim. Behav. 35: 844–855.

LAVIGNE, D.M. 1976. Counting harp seals with ultraviolet photography. Polar Rec. 18: 269–277.

LAVIGNE, D.M., S. INNES, AND W.W. BARCHARD. 1979. Interpretation and evaluation of harp seal census data. Polar Rec. 19: 381–405.

LAVIGNE, D.M., S. INNES, W. BARCHARD, AND W.G. DOUBLEDAY. 1980. The 1977 census of Northwest Atlantic harp seals, *Pagophilus groenlandicus*. Int. Comm. Northwest Atl. Fish. Sel. Pap. 6: 55–70.

LAVIGNE, D.M., S. INNES, K. KALPAKIS, AND K. RONALD. 1982. An aerial census of western Atlantic harp seals (*Pagophilus groenlandicus*) using ultraviolet photography. Int. Comm. Northwest Atl. Fish. Res. Doc. 75/144, Ser. 3717: 10 p.

LETT, P.F., AND T. BENJAMINSEN. 1977. A stochastic model for the management of the northwestern Atlantic harp seal population. J. Fish. Res. Board Can. 34: 1155–1187.

MYERS, R.A., AND W.D. BOWEN. 1989. Estimating bias in aerial surveys for harp seal pup production. J. Wildl. Manage. 53: 361–372.

NI, I-H., G.B. STENSON, AND H. RIPLEY. 1988. Evaluation of ultraviolet photography in the detection of seals, p. 96–107. *In* I. Ohnuki and V. Zsilinszky [ed.] Proc. Joint and Special Sessions on the Review of Advances in Remote Sensing Technology for Forestry. International Congress of Photogrammetry and Remote Sensing, July 6–7, 1988, Kyoto, Japan.

ROFF, D.A., AND W.D. BOWEN 1983. Population dynamics and management of the Northwest Atlantic harp seal. Can. J. Fish. Aquat. Sci. 40: 919–932.

ROFF, D.A., AND W.D. BOWEN. 1986. Further analysis of population trends in the northwest Atlantic harp seal (*Phoca groenlandica*) from 1967 to 1985. Can. J. Fish. Aquat. Sci. 43: 553–564.

SERGEANT, D.E. 1965. Migration of harp seals *Pagophilus groenlandicus* (Erxleben) in the northwest Atlantic. J. Fish. Res. Board Can. 22: 433–464.

SERGEANT, D.E. 1971. Calculation of harp seal production in the western north Atlantic. Int. Comm. Northwest Atl. Fish. Redbook 1971, Part III, Res. Doc. 71/7: 157–183.

SERGEANT, D.E. 1975. Estimating numbers of harp seals. Rapp. P.-v. Réun. Cons. int. Explor. Mer 169: 274–280.

SERGEANT, D.E., AND H.D. FISHER. 1960. Harp seal populations in the western North Atlantic from 1950 to 1960. Fish. Res. Board Can. (Arctic Unit, Montreal, Que.) Circ. No. 5: 58 p.

STEWART, R.E.A. 1987. Behavioral reproductive effort of nursing harp seals, *Phoca groenlandicus*. J. Mammal. 68: 348–358.

STEWART, R.E.A., AND D.M. LAVIGNE. 1980. Neonatal growth of northwest Atlantic harp seals, *Pagophilus groenlandicus*. J. Mammal. 61: 670–680.

WINTERS, G.H. 1978. Production, mortality, and sustainable yield of northwest Atlantic harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 35: 1249–1261.

# Seasonal Variation in Plasma Nonesterified Fatty Acids of Lake Sturgeon (*Acipenser fulvescens*) in the Vicinity of Hydroelectric Facilities

R.S. McKinley[1]

*Ontario Hydro Research, 800 Kipling Avenue, Toronto, ON M8Z 5S4, Canada*
*and*
*Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada*

T.D. Singer

*Department of Biochemistry, University of Toronto, Hospital for Sick Children, Toronto, ON M5S 1X8, Canada*

J.S. Ballantyne

*Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada*

and G. Power

*Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada*

McKinley, R.S., T.D. Singer, J.S. Ballantyne, and G. Power. 1993. Seasonal variation in plasma nonesterified fatty acids of lake sturgeon (*Acipenser fulvescens*) in the vicinity of hydroelectric facilities. Can. J. Fish. Aquat. Sci. 50: 2440–2447.

To establish the effects of hydroelectric generation on the health of lake sturgeon (*Acipenser fulvescens*), seasonal variations in plasma nonesterified fatty acids (NEFAs) upstream and downstream from hydroelectric stations were measured over a 2-yr period. Plasma NEFA profiles were also compared up- and downstream of the stations for differences in utilization of individual NEFA species as substrates for lipid oxidation. Significantly higher levels of total plasma NEFA were found in lake sturgeon upstream (2355 ± 395.9 nmol/mL) compared with those downstream (798 ± 133.5 nmol/mL) of the generating stations during the spring. The NEFA profiles for several key fatty acid species differed significantly among seasons up- and downstream of the facilities. In particular, during spring and summer, the levels of oleic acid (18:1*n*9) were highest upstream of the stations and levels of a polyunsaturated fatty acid, docosahexaenoic acid (22:6*n*3), were higher below rather than above the stations. The differences in plasma NEFA concentration may be attributed to altered nutritional status due to the varying flow regime located downstream of the hydroelectric stations.

Afin d'établir les effets de la production d'hydroélectricité sur la santé de l'esturgeon jaune (*Acipenser fulvescens*), on a mesuré, sur une période de 2 ans, les fluctuations saisonnières d'acides gras non estérifiés dans le plasma, en amont et en aval de stations hydroélectriques. Les profils de ces acides dans le plasma ont aussi été comparés, en amont et en aval des stations, pour ce qui est des différences d'utilisation des acides gras non estérifies pour chaque espèce comme substrat pour l'oxydation des lipides. On a relevé des teneurs significativement plus élevées d'acides gras non estérifiés chez l'esturgeon jaune fréquentant les zones en amont (2355 ± 395,9 nmol/mL) comparativement à ceux des zones situées en aval (798 ± 133,5 nmol/mL) des stations de production au printemps. Les profils des acides gras non estérifiés de plusieurs espèces clés d'acides gras présentaient des différences inportantes d'une saison à l'autre chez les poissons fréquentant les zones en amont et les zones en aval des installations. En particulier, au printemps et en été, les teneurs en acide oléique (18:1*n*9) étaient plus élevées chez les poissons en amont des stations et les teneurs en acide gras polyinsaturé, acide docosahexaénoïque (22:6*n*3), étaient plus élevées dans les zones en aval qlue dans les zones en amont des stations. Les différences de teneur en acide gras non estérifié dans le plasma peuvent être attribuées à une modification de l'alimentation due à la variation du débit en aval des stations hydroélectriques.

*Received October 29, 1992*
*Accepted May 31, 1993*
*(JB676)*

*Reçu le 29 octobre 1992*
*Accepté le 31 mai 1993*

Lake sturgeon (*Acipenser fulvescens*) is a highly prized resource for both sport and subsistence fishing by aboriginal people. Unfortunately, life history characteristics such as late sexual maturity and intermittent spawning have made the species particularly vulnerable to overfishing. As a result, the high exploitation rates of past commercial fishing practices have led to a decline in lake sturgeon in many parts of their native range (Harkness and Dymond 1961; Brousseau

[1]Author to whom reprint requests should be addressed.

1987). Today, lake sturgeon in Ontario are common only in rivers and lakes north of Lake Superior and the status of these populations is unknown.

One potential threat to lake sturgeon is the hydroelectric development of northern rivers. The power peaking mode of operation of some stations typically produces a diel cycle of water level fluctuations downstream of a station. Because the cycle is a function of demand, flow is generally high during the day and low during the night. The influence of this cycle on water flow is reflected downstream by periodic dewatering of river margins and changes in water velocity. The detrimental effects of these short-term fluctuations on the standing crop and community structure of the benthic community have also been documented in several studies (Fisher and LaVoy 1972; Fraser 1972; Trotzky and Gregory 1974; Gislason 1985). The subsequent effect on a benthic feeder such as lake sturgeon is not known but is likely to be reflected in its nutritional condition.

Procedures to evaluate nutritional condition of fish have included a Fulton's condition factor (Ricker 1975), an RNA/DNA index (Steinhart and Eckmann 1992), and the use of proteolytic enzymes (Pedersen et al. 1987). Alternatively, a biochemical index, reflective of nutritional history, and based on a measure of energetically available substrates (i.e. lipids), may best reflect overall metabolic activity. Plasma nonesterified fatty acids (NEFAs) are the most active form of lipid in the blood of vertebrates. The presence of specific NEFAs in the blood of teleosts indicates the extent to which they are mobilized and utilized as a source of fuel (Henderson and Tocher 1987). Changes in total NEFA levels can be used to determine differences in nutritional status of fish. Levels of individual fatty acids also provide further insight into their relative importance to overall metabolic activity. Similar approaches have been used to evaluate the mobilization and utilization of NEFAs in teleosts (Zammit and Newsholme 1979; Jezierska et al. 1982; Moon 1983), but only recently has a primitive, nonteleost species such as lake sturgeon been shown to possess a similar capacity to utilize NEFAs in their metabolism (Singer et al. 1990). To our knowledge, no previous study has reported seasonal changes in plasma NEFAs for any fish. The objectives of the present study were to examine the seasonal composition of plasma NEFAs in lake sturgeon and to use the NEFA profile from individuals collected upstream and downstream of hydroelectric facilities to establish the effects of these facilities on the nutritional status of lake sturgeon.

## Materials and Methods

### Study Site

The study was conducted over a 2-yr period (1989 and 1990) on the Mattagami River upstream and downstream of four hydroelectric facilities located approximately 75 km northeast of the town of Kapuskasing, Ont. (Fig. 1). Sampling locations were restricted to an area 21 km above and below the four stations.

The positions of the four generating stations (GS) located within the study area are shown in Fig. 1. The Smoky Falls GS is operated as a run-of-the river station with a total capacity of 200 $m^3/s$. The three remaining stations are peaking stations that exhibit a diel cycle and operated for approximately 5 h per day. These stations have capacities that range from 481 to 522 $m^3/s$. The different operating patterns cause the Smoky Falls, Harmon, and Kipling headpond levels to fluctuate daily. The Smoky Falls headpond is drawn down approximately 3 m to receive the peak discharge from Little Long GS. Similarly, both the Harmon and Kipling headponds must be drawn down to accommodate the discharge from Smoky Falls GS. With the exception of the Little Long GS headpond, there is little storage capacity along this stretch of the river. Retention times (reservoir volume divided by mean flow) are estimated at about 6 d for Little Long GS headpond, less than 8 h for Smoky GS headpond, 8 h for Harmon GS headpond, and 1 d for Kipling GS headpond. Differences in water temperature between downstream of Kipling GS and upstream of Little Long GS are not apparent.

The only time of the year when the mode of operation switches to run-of-the-river is during the spring when inflow greatly exceeds plant capacities. During this time, excess water is spilled through the Adam Creek control structures. A peaking operation governs water flows and levels in the Mattagami River during winter, midsummer, and early fall.

### Sample Collection

Upstream fish were collected south of Little Long GS, and downstream fish were collected north of Kipling GS. No sampling was conducted in between stations.

A similar sampling effort was conducted up and downstream of the four stations. Adult lake sturgeon were collected using gill nets (15.2 × 1.8 m panels, 20-cm stretched mesh). Adults were collected only during ice-free conditions. A blood sample, minimum of 0.5 mL, was collected by caudal puncture using heparinized syringes. The whole-blood samples were subsequently centrifuged and the plasma collected and frozen (–20°C). In most cases, fish were released unharmed after sampling.

### Sample Analysis

NEFAs in plasma samples were assayed by the method of Lepage and Roy (1988). Selective methylation of NEFAs was achieved in the presence of a methanol – acetyl chloride mixture (50:1). A known amount of internal standard (15 μg), heptadecanoic acid (17:0), was methylated along with plasma NEFAs. Plasma samples (150 μL) containing a known amount of internal standard and methanol – acetyl chloride (5 mL) were placed in glass tubes equipped with Teflon-lined caps. Tubes were maintained at 24–29°C with constant stirring for 45 min in a Reacti-Therm (Pierce, Rockford IL 61105) heating/stirring dry block. At the completion of methylation, a 6% potassium carbonate solution (3 mL) was added to the mixture followed by the addition of hexane (150 μL). Tubes were subsequently shaken and centrifuged for 10 min at 2000*g*. The hexane upper phase containing methyl esters of NEFAs was removed using a pipette and placed in a separate vial. Hexane was then evaporated under oxygen-free nitrogen gas and methyl esters were redissolved in 50 μL of carbon disulphide ($CS_2$). Two microlitres of $CS_2$, containing methyl esters, was injected into a gas chromatograph (Hewlett-Packard HP589011) equipped with a flame ionization detector. Fatty acid methyl esters were analyzed on a DB-225 megabore fused silica column (Chromatographic Specialities Inc., Brockville, Ont.) under an initial temperature of 60°C and a final temperature of 210°C using helium as the carrier gas. The identification of unknown fatty acid methyl esters was accomplished by comparing their retention times with known standards, and the absolute amounts of these fatty acids were determined with the aid of the internal standard, heptadecanoic acid (17:0).

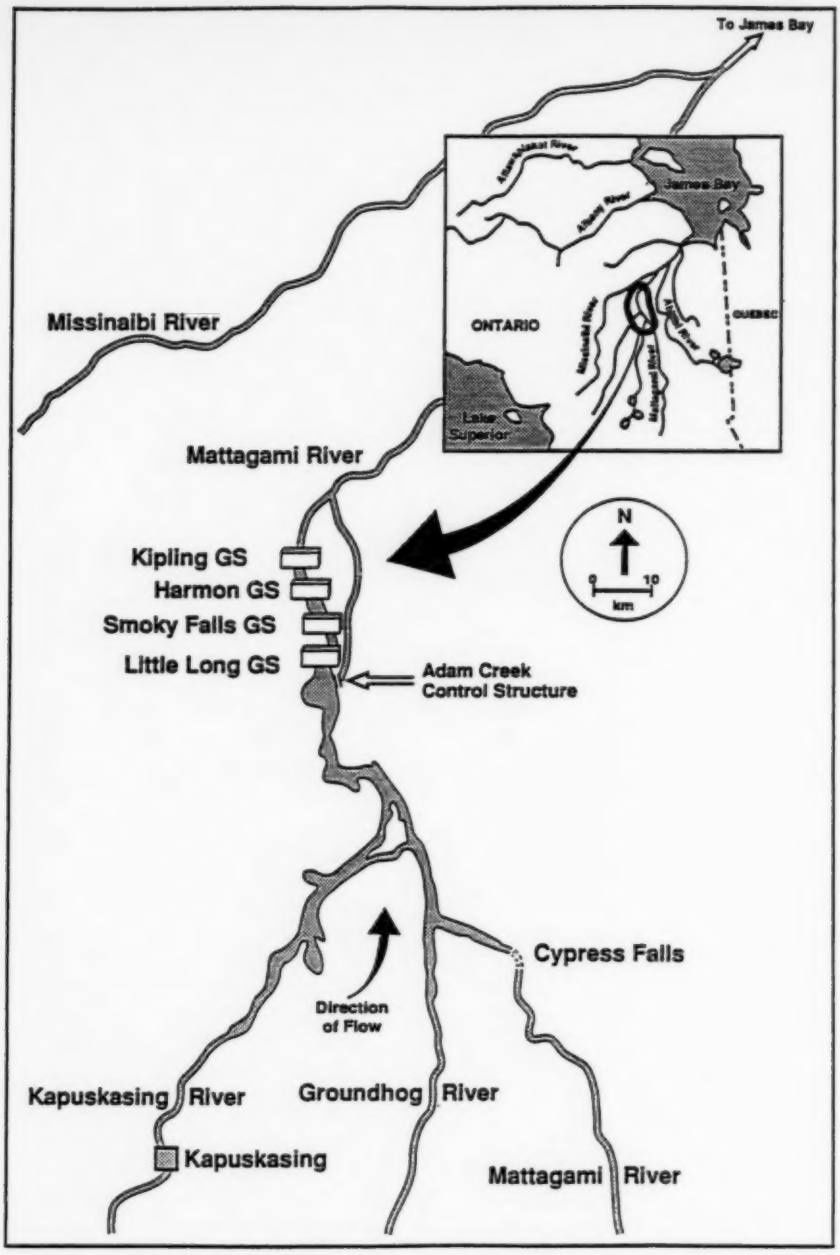



FIG. 1. Location of hydroelectric generating stations referred to in this study.

## Chemicals

With the following exceptions, chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.) and were of highest purity available. Lipid class standard was obtained from NU Chek Prep. Inc. (Elysian, Minn.) augmented with menhaden and borage oil extract. Solvents used in lipid extraction were obtained from Fisher Scientific (Toronto, Ont.).

## Statistical Analysis

Differences in total plasma fatty acids and major fatty acid classes over the two years and among seasons (spring, summer, and fall) for individuals collected upstream and downstream of the four hydroelectric stations were tested using ANOVA. Significant differences ($p < 0.05$) were further investigated using LSMEANS (Steel and Torrie 1980). Log-transformations of data

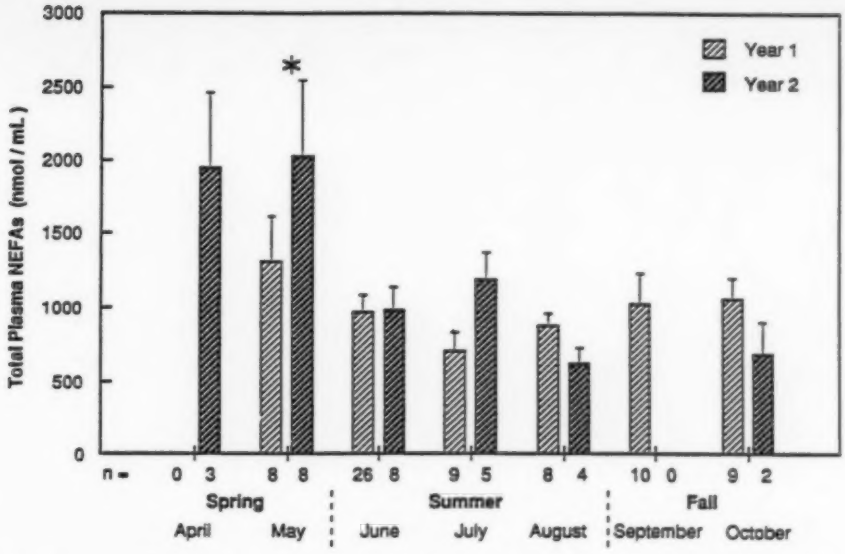



FIG. 2. Seasonal variation in plasma NEFAs for lake sturgeon collected over the two years. Values are means ± SEM. Significant differences in NEFAs are indicated by an asterisk ($p < 0.05$). Data presented are averages for both upstream and downstream fish.

were used in certain cases to meet assumptions of parametric tests (Steel and Torrie 1980).

## Results

Total plasma NEFAs over the two sampling years exhibited a similar seasonal pattern (Fig. 2). The sole exception was a significantly lower total NEFA level in May 1989 (1312 ± 295.7 nmol/mL) than in May 1990 (2036 ± 510.3 nmol/mL) ($p = 0.03$). Total plasma NEFAs for both years combined were significantly higher in spring (1719 ± 260.8 nmol/mL) than in either summer (915 ± 57.2 nmol/mL) or fall (1003 ± 114.0 nmol/mL) ($p < 0.05$).

For both upstream and downstream fish, significantly higher levels of total NEFAs were observed during the spring than either in summer or fall (Fig. 3). Partitioning of data by location revealed that total NEFAs were significantly higher in May for lake sturgeon collected upstream (2355 ± 395.9 nmol/mL) than for those sampled downstream of the hydroelectric facilities (798 ± 133.5 nmol/mL). Lake sturgeon upstream or downstream of the facilities exhibited similar total NEFAs during all other months.

Molar percent comparison of the major classes of fatty acid (saturated, monounsaturated, and polyunsaturated) on a seasonal basis revealed differences in the restructuring of the lipid composition in fish sampled upstream versus downstream of the facilities (inset, Fig. 3). In the spring, upstream fish had a significantly higher percentage of monounsaturates (50.17 ± 1.92%) than downstream fish (38.82 ± 1.54%) ($p < 0.05$). The opposite was observed for polyunsaturated fatty acids (upstream 27.68 ± 2.27% versus downstream 36.89 ± 2.06%; $p < 0.05$). Percentage of saturated fatty acids was not significantly different in upstream versus downstream fish in both spring and summer. Similar to spring, upstream fish during the summer exhibited a significantly higher percentage of monounsaturates (51.22 ± 0.72%) versus downstream fish (44.53 ± 2.32%) ($p < 0.01$) and at the same time a significantly lower percentage of polyunsaturated fatty acids (upstream 23.99 ± 0.67%) than downstream fish (29.63 ± 2.45%) ($p < 0.01$). Only during the fall was a significant difference in saturated fatty acids observed between upstream and downstream fish (22.06 ± 0.84 and 27.98 ± 1.54%, respectively; $p < 0.05$).

Further analysis of the polyunsaturated fatty acids (PUFA) revealed a significantly lower percentage of the *n*3 series fatty acids in upstream (10.5 ± 1.08%) versus downstream fish (18.2 ± 2.06%) during the spring. A similar trend was observed during the summer for upstream lake sturgeon (9.2 ± 0.4%) versus downstream lake sturgeon (13.7 ± 1.9%). Levels of *n*3 fatty acids were similar during the fall. There were no differences in the percentage of *n*6 series between upstream and downstream fish in any season.

Over the two years of study, 10 of the 23 individual fatty acids measured contributed more than 80% to the total plasma NEFA: 14:0, 16:0, 16:1, 18:0, 18:1*n*9, 18:2*n*6, 20:2*n*6, 20:4*n*6, 20:5*n*3, and 22:6*n*3. The predominant NEFA in the plasma of lake sturgeon over all seasons was the monoene 18:1*n*9 (33.5%). The other major fatty acids were two saturated acids, 14:0 (3%) and 16:0 (16.7%), one monoene, 16:1 (12.4%), and three polyunsaturates, 20:4*n*6 (7.5%) 20:5*n*3 (4%), and 22:6*n*3 (5.8%).

Of the major fatty acids, significant differences between upstream and downstream samples were only observed for the monoenes 16:1 and 18:1*n*9 and for the two polyunsaturates 20:5*n*3 and 22:6*n*3. 18:1*n*9 was significantly higher in lake sturgeon collected upstream of the hydroelectric facilities in both spring and summer (37.5 and 25.3%, respectively). Levels between upstream and downstream were similar during the fall (Fig. 4). Of the two saturated fatty acids 14:0 and 16:0, only the former was found to be significantly higher in lake sturgeon

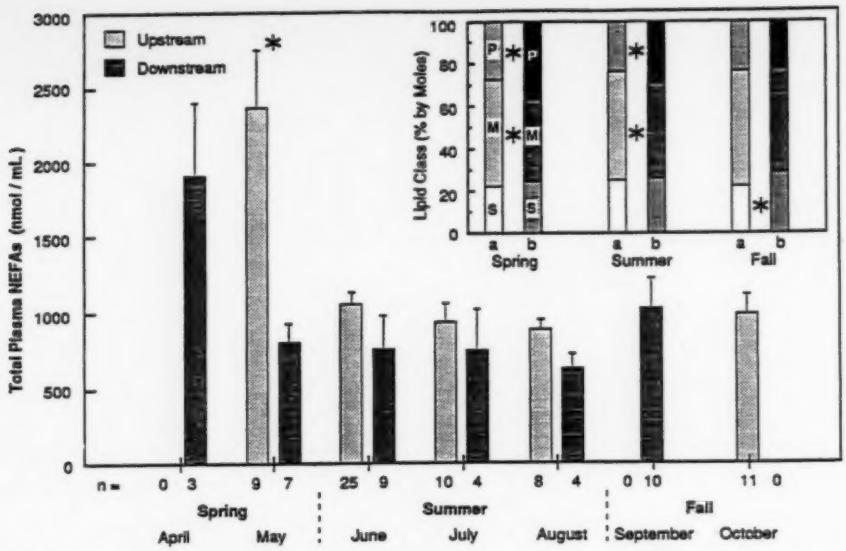



FIG. 3. Seasonal variation in plasma NEFAs for lake sturgeon collected upstream and downstream of the hydroelectric facilities. Major classes of lipids throughout the seasons are also shown between locations above (a) and below (b) the facilities (inset); P, polyunsaturates; M, monounsaturated, S, saturates. Values are means ± SEM. Significant differences between upstream and downstream fish as well as among classes are indicated by an asterisk ($p < 0.05$).

collected downstream of the facilities, but only during the summer. Among the three dominant polyunsaturates, only 20:4*n*6 exhibited similar levels in the plasma of lake sturgeon collected upstream and downstream of the hydroelectric facilities over all three seasons. 22:6*n*3 was significantly higher in plasma samples collected downstream of the stations, but only in the spring and summer. Only during the spring was a significantly higher 20:5*n*3 detected in the plasma of lake sturgeon collected downstream of the stations.

## Discussion

The data presented demonstrate the usefulness of using plasma NEFAs as indices of the health of lake sturgeon above and below hydroelectric facilities. Most studies examining lipid composition in fish have concentrated on relatively inert lipids (triglycerides and phospholipids) in selective tissues and individual organs of teleosts (see review by Greene and Selivonchick 1987). These studies have shown that fish contain high levels of long-chain unsaturated acids, but the importance of chain length and degree of saturation of individual fatty acid species as metabolic substrates for β-oxidation cannot be determined from such studies. Only recently have studies of more metabolically active plasma NEFAs demonstrated obvious preferences among fish, including primitive fish such as lake sturgeon and bowfin (*Amia calva*), for the mobilization of individual plasma lipids for energy metabolism (Singer et al. 1990, Singer and Ballantyne 1991).

Only 5–10% of the total plasma lipid is in the form of NEFAs bound to a carrier protein, albumin. Despite NEFAs minor contribution to the total plasma lipid, these fatty acids represent the most active form of lipids available for energy metabolism in the blood of vertebrates (Henderson and Tocher 1987). The importance of lipid as an energy source for metabolism in fishes has been shown for seasonal events such as gonadal maturation and migration (Moon 1983; Takima et al. 1985; van den Thillart 1986, respectively) The present study indicates that lake sturgeon selectively mobilize different NEFAs depending on the season. In general, elevated plasma NEFA levels are interpreted as an increased mobilization of lipid to supply energy for a variety of possible activities.

We found the highest levels of NEFAs in lake sturgeon during their spring migration and spawning period. Although feeding occurs in the spring before spawning, lake sturgeon have been reported not to feed during spawning (Houston 1987), requiring a mobilization of lipid stores during this period. This is reflected in the elevated total plasma NEFAs in the spring. Well-fed upstream fish would have higher lipid reserves to mobilize than fish downstream, and hence, total plasma NEFAs are higher in spring in upstream fish. The relationship between lipid metabolism and gonadal maturation has been reported in several fish species (European flounder (*Platichthys flesus*), Petersen and Emmersen 1977; plaice (*Pleuronectes platessa*), White et al. 1986; sea bass (*Dicentrarchus labrax*), Fernandez et al. 1989). Typically, these studies correlated increased total plasma lipids with gonadal maturation. The subsequent drop in utilization of NEFAs in the summer and early fall may have also been a consequence of an observed decrease in locomotory activity for lake sturgeon in the field as water temperatures approach 19°C (McKinley and Power 1991). Plisetskaya (1980) and Henderson and Tocher (1987) have suggested that plasma fatty acid levels are directly related to locomotory activity.

The specific fatty acids comprising the lipids of fish are greatly influenced by diet. Numerous dietary studies have shown that the fatty acid composition of tissue lipids in freshwater fish is greatly influenced by the patterns of fatty acids in their dietary lipid (see review by Henderson and Tocher 1987). Evidence that a transfer of fatty acids from one tropic level to another can occur

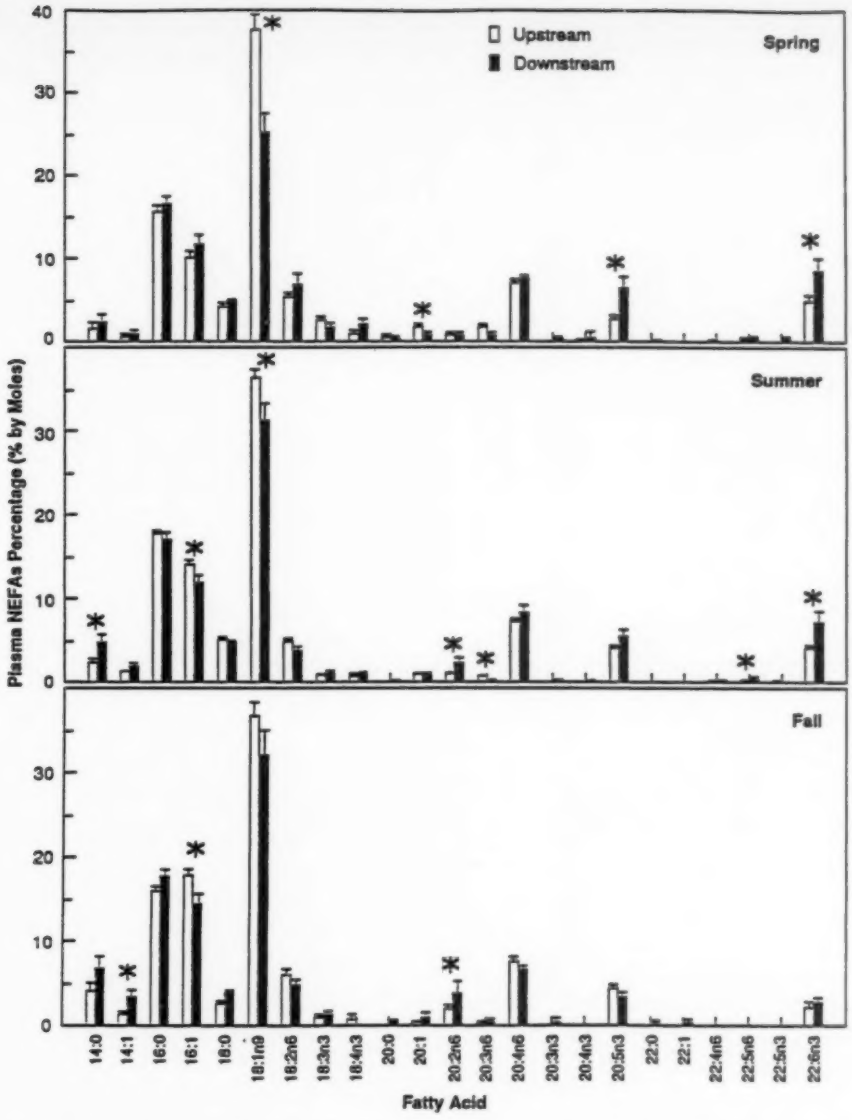



FIG. 4. Seasonal variation in fatty acid composition of lake sturgeon collected upstream and downstream of the hydroelectric facilities. Values are means ± SEM. Significant differences between upstream and downstream fish are indicated by an asterisk ($p < 0.05$).

has been shown by Fraser et al. (1989). One factor particularly indicative of dietary fatty acids is the *n*3/*n*6 ratio of fatty acids. The *n*3/*n*6 ratio measured in this study was significantly higher in downstream fish in both spring and summer, 1.26 ± 0.23 versus 0.66 ± 0.05 and 0.96 ± 0.17 versus 0.68 ± 0.03, respectively. These values for lake sturgeon are at the low end of the range suggested for freshwater fish (0.5–3.8, Henderson and Tocher 1987).

As indicated above, the ability to acquire and store lipids is directly related to the availability of prey. Gislason (1986) credited high condition factor for several young-of-the-year salmonids to the improved insect density found in stable flow conditions. The high levels of plasma NEFAs of lake sturgeon upstream in the spring may indicate a more diverse and abundant prey before spawning than may be found under the more stable water levels that occur above than below the stations where the benthic community is subjected to diel fluctuations in water flow. The improved dietary status of upstream fish results in an enhanced mobilization of NEFA during the spawning period when activity increases and feeding stops.

The diversity of prey items in the diet can also be expected to influence the overall NEFA profile. Preliminary analysis of the stomachs of a few sacrificed lake sturgeon indicated that upstream fish had a much different diet over the three seasons than fish downstream of the stations. Upstream, their primary prey items were insects, notably *Hexagenia* sp. Fatty acid species of

TABLE 1. Ratio of docosahexaenoic acid to oleic acid for lake sturgeon collected above and below the hydroelectric generating stations for each of the three seasons. Significant differences between sites for each season are indicated by an asterisk.

| Spring* | | Summer* | | Fall | |
|---|---|---|---|---|---|
| Above | Below | Above | Below | Above | Below |
| 13.9 ± 2.0 | 33.5 ± 5.6 | 11.9 ± 0.8 | 27.3 ± 5.1 | 6.6 ± 1.7 | 10.8 ± 3.7 |

18:2*n*6, 18:3*n*3, and to a lesser extent 20:4*n*6 and 20:5*n*3 are known to predominate in aquatic insects, regardless of life stage (Henderson and Tocher 1987). Downstream of the station, lake sturgeon fed primarily on small fish and crayfish over the three seasons. The plasma NEFA profiles of these fish probably reflected to some extent the fatty acid profiles of their limited prey. While very few studies have been conducted, freshwater fish have significantly higher levels of 22:6*n*3 than aquatic insects (see review by Henderson and Tocher 1987). Further studies of the fatty acid composition of the organisms comprising the diet of lake sturgeon above and below the stations are required to verify this hypotheses. The difference in availability of prey items between locations up- versus downstream of the stations is likely due to the ability of the prey to cope with variations in water flow and levels. These variations can include 34% of the available aquatic habitat being dewatered for at least 19 km north of the last generating station (Carson et al. 1991). Only the most mobile of prey (i.e., fish) are likely to survive in situ.

The NEFA profile for up- and downstream fish varied seasonally. The monoenoic fatty acids contributed most to the difference between the two locations. The preferential mobilization of monosaturates, particularly long-chain fatty acids (C > 18), for use in energy metabolism has been reported in several studies (rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*), Jezierska et al. 1982; Atlantic cod (*Gadus morhua*), Takima et al. 1985; antarctic fish, Crockett et al. 1988; Sidell 1991; arctic fish, J.S. Ballantyne et al., unpublished data). For lake sturgeon kept in captivity, high levels of monoenes, particularly oleic acid (18:1*n*9), in the plasma NEFAs have also been observed (Singer et al. 1990). Our study also revealed high levels of oleic acid, but its percent contribution to the overall NEFA profile was considerably less than reported by Singer et al. (1990). The reduced importance of oleic acid between the two studies is due to the greater contribution made by several saturates and polyunsaturates to the overall profile. The preferential mobilization of monoenes over saturated fats has been found in arctic (J.S. Ballantyne et al., unpublished data) and antarctic fish species (Sidell 1991). In the present study, saturated fats were only found to be high during the fall in lake sturgeon downstream of the generating facilities. Jezierska et al. (1982) found similar energy production from monounsaturates and saturates and suggested that factor(s) other than energy content are determining their pattern of mobilization.

Among the polyunsaturates, eicosapentaenoic acid (20:5*n*3) and docosahexaenoic acid (22:6*n*3) contributed most to the difference in NEFA profiles between up- and downstream lake sturgeon populations. The utilization of these fatty acids may have compensated for the low downstream levels of oleic acid. The mobilization of docosahexaenoic acid is particularly surprising because this fatty acid species has been deemed important (belonging to the *n*3 series) due to its unique physical properties for the maintenance of biological membranes (Bell et al. 1986). Furthermore, little of this fatty acid (3%) is derived directly from dietary items (Hazel 1979), and total levels are likely a consequence of desaturation and elongation of shorter, less saturated *n*3 fatty acids in the diet (Fraser et al. 1989). The energetic cost to the animal of desaturation and elongation is significant (Jezierska et al. 1982), and therefore, it is to the advantage of the animal not to utilize long-chain PUFAs. It is probably for this reason that 22:6*n*3 is generally retained, even in starving fish (Henderson and Tocher 1987). The significantly lower docosahexaenoic acid below the generating stations may indicate a more restricted intake of *n*3 fatty acids as indicated earlier and/or an extreme metabolic condition requiring the mobilization and oxidation of all fatty acids.

The comparison of the ratio between a preferred metabolic substrate, oleic acid, and a fatty acid not normally oxidized, docosahexaenoic acid, may offer a simple method for predicting differences in energy metabolism between locations. A comparison of the ratios of 22:6*n*3/18:1*n*9 showed that significant differences were detected between locations during the spring and summer (Table 1). The ratio was remarkably consistent at the upstream site among the three seasons and suggests that it does not normally vary. The significantly higher ratios downstream of the stations indicated that the lake sturgeon are mobilizing 22:6*n*3 and are likely metabolically challenged. Such an approach has been previously used for chum salmon (*Oncorhynchus keta*) where the ratio of preferred and nonpreferred metabolic substrates has been shown to decrease with the increasing costs of migration (Takahashi et al. 1985). Such an approach may offer a simple and direct method of evaluating potential anthropogenic effects on energy metabolism in fish.

The one polyunsaturated fatty acid that did not differ between up- and downstream sites was arachidonic acid (20:4*n*6). This fatty acid is important as a major precursor for prostaglandin synthesis (Greene and Selivonchick 1987). Because prostaglandins have been reported to play several roles in fish reproduction, including ovulation and behavioral changes associated with spawning (Stacey and Peter 1979; Stacey and Goetz 1982, respectively), it is to the advantage of the fish to retain a fatty acid important for prostaglandin synthesis.

Although significant differences in plasma NEFAs were found between lake sturgeon situated above and below the hydroelectric facilities, we can at this point only speculate as to the exact cause. The most likely hypothesis is that the difference in condition, based on lipid composition, observed between upstream and downstream lake sturgeon is a consequence of a variable water flow on the benthic macroinvertebrate community with concomitant effects on the diet of the lake sturgeon. Both the quantity and type of lipid available to the fish above and below the facilities likely differ. Further study is required to examine the importance of the diversity and abundance of prey items between a regulated and unregulated river in the lipid

composition of lake sturgeon. The lipid composition of lake sturgeon above the facilities may also be a consequence of an improved but artificial habitat created by the hydroelectric inpoundment. An important question to be answered is what is the significance of a high docosahexaenoic acid to oleic acid ratio in lake sturgeon below the stations.

## Acknowledgements

We are indebted to Messers H.E. Kowalyk and R.W. Sheehan for their assistance in the collection of samples. We also wish to thank Drs. J.S. Griffiths and A.M. Sills and Mr. R. Evans for their critical review of the manuscript. Funding for this study was provided by Ontario Hydro Research.

## References

BELL, M.V., R.J. HENDERSON, AND J.R. SARGENT. 1986. The role of polyunsaturated fatty acids in fish. Comp. Biochem. Physiol. 83B: 711–719.

BROUSSEAU, C.S. 1987. The lake sturgeon (*Acipenser fulvescens*) in Ontario, p. 2–9. *In* C.H.Olver [ed.] Proc. Workshop on the lake sturgeon (*Acipenser fulvescens*). Ont. Fish. Rep. Ser. No. 23.

CARSON, R.A., A.P. SANDILANDS, AND R.R. EVANS. 1991. Hydroelectric generating stations — Mattagami River. Mattagami river hydraulic studies and impacts on fisheries habitat. Ont. Hydro Rep. No. 90367: 53 p.

CROCKETT, E.L., B.D. SIDELL, AND W.R. DRIEDZIC. 1988. Metabolic fuel preference in muscle tissues from an Antarctic fish, *Notothenia gibberifrons*. Am. Zool. 28: 42A.

FERNANDEZ, J., J. GUTIERREZ, M. CARRILLO, S. ZANUY, AND J. PLANAS. 1989. Annual cycle of plasma lipids in sea bass, *Dicentrarchus labrax* L.: effects of environmental conditions and reproductive cycle. Comp. Biochem. Physiol. 93A: 407–412.

FISHER, S.G., AND A. LAVOY. 1972. Differences in littoral fauna due to fluctuating water levels below a hydroelectric dam. J. Fish. Res. Board. Can. 29: 1472–1476.

FRASER, J.C. 1972. Regulated discharge and the stream environment, p. 263–285. *In* R.T. Oglesby, C.A. Carlson, and J.A. McCann [ed.] River ecology and man. Academic Press, New York, N.Y.

FRASER, A.J., J.R. SARGENT, J.C. GAMBLE, AND D.D. SEATON. 1989. Formation and transfer of fatty acids in an enclosed marine foood chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar. Chem. 27: 1–18.

GISLASON, J.C. 1985. Aquatic insect abundance in a regulated stream under fluctuating and stable diel flow patterns. N. Am. J. Fish Manage. 5: 39–46.

GREENE, D.H.S., AND D.P. SELIVONCHICK. 1987. Lipid metabolism in fish. Prog. Lipid Res. 26: 53–85.

HARKNESS, W.J.K., AND J.R. DYMOND. 1961. The lake sturgeon: the history of its fishery and problems of conservation. Ontario Department of Lands and Forests, Fish and Wildlilfe Branch, Toronto, Ont. 121 p.

HAZEL, J.R. 1979. Influence of thermal acclimation on membrane lipid composition of rainbow trout liver. Am. J. Physiol. 236: R91–R101.

HENDERSON, R.J., AND D.R. TOCHER. 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26: 281–347.

HOUSTON, J.J. 1987. Status of the lake sturgeon, *Acipenser fulvescens*, in Canada. Can. Field-Nat. 101: 171–185.

JEZIERSKA, B., J.R. HAZEL, AND S.D. GERKING. 1982. Lipid mobilization during starvation in the rainbow trout, *Salmo gairdneri* Richardson, with attention to fatty acids. J. Fish Biol. 21: 681–692.

LEPAGE, G., AND C.C. ROY. 1988. Specific methylation of plasma nonesterified fatty acids in a one-step reaction. J. Lipid Res. 29: 227–235.

MCKINLEY, R.S., AND G. POWER. 1991. Measurement of activity and oxygen consumption for adult lake sturgeon (*Acipenser fulvescens*) in the wild using radio transmitted EMG signals. *In* I.G. Priede and S.M. Swift. [ed.] Wildlife telemetry, remote monitoring and tracking of animals. Ellis Horwood, West Sussex, England. 708 p.

MOON, T.W. 1983. Metabolic reserves and enzyme activities with food deprivation in immature American eels, *Anguilla rostrata* (leSueur). Can. J. Zool. 61: 802–811.

PEDERSEN, B.H., E.M. NILSSEN, AND K. HJELMELAND. 1987. Variations in the content of trypsin and trypsinogen in larval herring (*Clupea harengus*) digesting copepod nauplii. Man. Biol. 94: 171–181.

PETERSEN, I.M., AND B.K. EMMERSEN. 1977. Changes in serum glucose and lipids and liver glycogen and phosphorylase during vitellogenesis in nature in the flounder (*Platichthys flesus*). Comp. Biochem. Physiol. 58B: 167–171.

PLISETSKAYA, E. 1980. Fatty acid levels in blood of cyclostomes and fish. Environ. Biol. Fishes 5: 273–290.

RICKER, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 386 p.

SIDELL, B.D. 1991. Physiological roles of high lipid content in tissues of Antarctic fish species, p. 220–231. *In* G. di Prisco, B. Maresca, and B. Tota [ed.] Biology of Antarctic fish. Springer-Verlag, Berlin, Germany.

SINGER, T.D., AND J.S. BALLANTYNE. 1991. Metabolic organization of a primitive fish, the bowfin (*Amia calva*). Can. J. Fish. Aquat. Sci 48: 611–618.

SINGER, T.D., V.G. MAHADEVAPPA, AND J.S. BALLANTYNE. 1990. Aspects of the energy metabolism of lake sturgeon, *Acipenser fluvescens*, with special emphasis on lipid and ketone body metabolism. Can. J. Fish. Aquat. Sci. 47: 873–881.

STACEY, N.E., AND F.W. GOETZ. 1982. Role of prostaglandins in fish reproduction. Can. J. Fish. Aquat. Sci. 39: 92–98.

STACEY, N.E., AND R.E. PETER. 1979. Central action of prostaglandins in spawning behaviour of female goldfish. Physiol. Behav. 22: 1191–1196.

STEEL, R.G.D., AND J.H. TORRIE. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill Inc., New York. N.Y. 633 p.

STEINHART, M., AND R. ECKMANN. 1992. Evaluating the nutritional condition of individual whitefish (*Coregonus* spp.) larvae by the RNA/DNA ratio. J. Fish Biol. 40: 791–799.

TAKAHASHI, K., M. EGI, AND K. ZAMA. 1985. Changes in molecular species of fish muscle phosphatidylcholine of chum salmon during migration. Bull. Jpn. Soc. Sci. Fish. 51: 1487–1493.

TAKIMA, K., R. MALCOLM LOVE, AND G.L. SMITH. 1985. Selectivity in mobilization of stored fatty acids by maturing cod, *Gadus morhua* L. Comp. Biochem. Physiol. 80B: 713–718.

TROTZKY, H.M., AND R.W. GREGORY. 1974. The effects of water flow manipulation below a hydro-electric power dam on the bottom fauna of the upper Kennebec River, Maine. Trans. Am. Fish. Soc. 103: 318–324.

VAN DEN THILLART, G. 1986. Energy metabolism of swimming trout (*Salmo gairdneri*). J. Comp. Physiol. B 156: 511–520.

WHITE, A., T.C. FLETCHER, AND J.A.POPE. 1986. Seasonal changes in serum lipid composition of the plaice, *Pleuronectes platessa* L. J. Fish Biol. 28: 595–606.

ZAMMIT, V.A., AND E.A. NEWSHOLME. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J. 184: 313–322.

# Importance of Condition Factor and the Influence of Water Temperature and Photoperiod on Metamorphosis of Sea Lamprey, *Petromyzon marinus*

J.H. Youson, J.A. Holmes, and J.A. Guchardi

*Division of Life Sciences, Scarborough Campus, University of Toronto, 1265 Military Trail, Scarborough, ON M1C 1A4, Canada*

J.G. Seelye

*United States Fish and Wildlife Service, Hammond Bay Biological Station, Millersburg, MI 49759, USA*

R.E. Beaver

*United States Fish and Wildlife Service, Ludington Biological Station, Ludington, MI 49431, USA*

J.E. Gersmehl

*United States Fish and Wildlife Service, Fisheries Assistance, Essex Junction, VT 05452, USA*

S.A. Sower

*Department of Biochemistry and Molecular Biology, University of New Hampshire, Durham, NH 03824, USA*

and F.W.H. Beamish

*Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada*

Youson, J.H., J.A. Holmes, J.A. Guchardi, J.G. Seelye, R.E. Beaver, J.E. Gersmehl, S.A. Sower, and F.W.H. Beamish. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. 50: 2448–2456.

The incidence of metamorphosis of larval sea lamprey, *Petromyzon marinus*, was strongly affected by water temperature but not photoperiod. In a 1991 experiment, the development of metamorphosing animals in 13°C water was retarded about 1 mo relative to animals metamorphosing at 21°C and to a population from the Chippewa River, Michigan; the minimum length, weight, and condition factor (CF) of metamorphosing experimental animals were 117 mm, 2.8 g, and 1.50, respectively, and only 4% metamorphosed at 13°C and 18.9% at 21°C. In 1992, with a population from the Great Chazy River, New York, 66% of the animals at 13°C and 84% at 21°C metamorphosed. The higher incidence of metamorphosis in 1992 is partly related to the use of larvae that were larger than the minima established in 1991. We predicted, using criteria defined below, that 74 and 72% of the animals at 13 and 21°C, respectively, would metamorphose. Our predictions were consistent with observations at 13°C and for five of seven replicate tanks at 21°C. We suggest that a presumptive metamorphosing sea lamprey in landlocked populations should be at least 120 mm long, weigh 3.0 g, and have a CF $\geq$ 1.50 and that these criteria must be used in conjunction.

L'apparition de la métamorphose des larves de la grande lamproie marine, *Petromyzon marinus*, était fortement touchée par la température de l'eau, mais pas par la photopériode. Lors d'une expérience menée en 1991, l'évolution de la métamorphose des animaux dans de l'eau à 13 °C a été retardée de 1 mo environ par rapport aux animaux qui se métamorphosait dans de l'eau à 21 °C et une population de la rivière Chippewa, Michigan; la longueur minimale, le poids et le coefficient de condition des animaux subissant une métamorphose expérimentale étaient respectivement de 117 mm, 2,8 g et 1,50 et seulement 4 % des animaux se sont métamorphosés à 13 °C et 18,9 % à 21 °C. En 1992, dans le cas d'une population de la rivière Great Chazy, New York, 66 % des animaux se sont métamorphosés à 13 °C et 84 % à 21 °C. La fréquence plus élevée de métamorphose en 1992 est liée en partie à l'utilisation de larves qui étaient plus grosses que la taille minimale établie en 1991. À partir des critères définis ci-dessous, nous prévoyons que respectivement 74 et 72 % des animaux placés respectivement dans de l'eau à 13 et 21 °C se métamorphoseront. Nos prévisions étaient conformes aux observations effectuées à 13 °C et pour cinq des sept bassins d'étude à 21 °C. Selon nous, dans des populations dulcicoles, une grande lamproie marine entamerait sa métamorphose à moins 120 mm de longueur, pour un poids de 3,0 g et avec un coefficient de condition de 1,50 et que ces critères devraient être utilisés conjointement.

*Received December 31, 1992*
*Accepted May 21, 1993*
*(JB745)*

*Reçu le 31 décembre 1992*
*Accepté le 21 mai 1993*

The larval period of landlocked sea lamprey, *Petromyzon marinus*, in the Great Lakes varies in duration from about 3 to 7 yr and is followed by a metamorphosis (transformation phase) which results in the development of a juvenile capable of parasitising teleost fish (Applegate 1950; Potter 1980). The time between the initiation of metamorphosis in landlocked sea lamprey (June–July) and the onset of feeding on teleosts varies from a minimum of 3–4 mo up to 10 mo (Applegate 1950; Potter 1980). Newly transformed animals emerge from the substrate and migrate into the Great Lakes in the fall or overwinter in the streams and migrate the following spring.

Lamprey metamorphosis is a highly programmed and synchronized sequence of developmental events (Youson 1980, 1988). Five stages of metamorphosis based on external features were first described for landlocked sea lamprey by Manion and Stauffer (1970). The external characters were reexamined in anadromous sea lamprey to accommodate early internal changes (Youson et al. 1977), resulting in an increase in the number of stages to seven (1, earliest; to 7, latest; Youson and Potter 1979). The seven stages are now generally used for all lamprey species (Potter et al. 1982).

Length is an important variable influencing the initiation of metamorphosis in lamprey with landlocked sea lamprey entering metamorphosis at a larger length than is the case with anadromous sea lamprey. The average length of metamorphosing animals in the Great Lakes is approximately 140 mm (Potter 1980) compared with 129 mm among animals from an anadromous population in New Brunswick (Potter et al. 1978). The smallest lengths at which the initiation of metamorphosis has been observed in landlocked and anadromous populations are 120 and 110 mm, respectively, and there is strong empirical evidence that animals smaller than these lengths do not enter metamorphosis (see Potter 1980; Smith and Tibbles 1980).

Metamorphosis is a nontrophic phase of immense physiological and morphological changes in sea lamprey (Potter 1980; Youson 1980), during which catabolism of lipid is their primary source of energy (Youson et al. 1979). Lipid levels of nonmetamorphosing larvae remain at about 4% of the live body weight until the larvae reach lengths comparable with that of animals during metamorphosis; lipid levels then increase to 14% of the live body weight prior to the onset of metamorphosis (Lowe et al. 1973; O'Boyle and Beamish 1977). Some animals of metamorphosing length may go through an interval of indeterminate length prior to metamorphosis called an "arrested growth" phase (Hardisty and Potter 1971a; Potter 1980; Youson 1988), during which the large lipid reserves are accumulated. This physiological conditioning is expressed by the addition of weight to the animal, as there is little or no increase in length while it is occurring (Hardisty and Potter 1971a; Potter 1980; Youson 1980). Therefore, both length and weight (size) are important criteria in preparation of animals for metamorphosis.

The relationship between length and body mass has been described as a condition factor (CF, where CF = weight (g)/(length (mm))$^3 \times 10^6$) for teleost fishes (Fulton 1911, cited in Ricker 1975) and was applied to sea lamprey in the larval period and during metamorphosis by Potter et al. (1978). Potter et al. (1978) showed that CF was significantly greater in metamorphosing stages 1 and 2 of anadromous sea lamprey than in nonmetamorphosing larvae of metamorphosing length (124–131 mm) and that CF declines progressively during metamorphosis. These differences in CF reflect the need to build up lipid levels in premetamorphic larvae and the utilization of lipid during metamorphosis (Lowe et al. 1973; O'Boyle and Beamish 1977; Youson et al. 1979; Youson 1988). Changes in the CF of landlocked sea lamprey during metamorphosis have not been studied.

The synchrony of metamorphosis in anadromous (Potter et al. 1978) and landlocked sea lamprey populations from the Great Lakes region (Manion and Stauffer 1970; Potter 1980) suggests that the initiation of metamorphosis at the end of the larval period may be sensitive to environmental cues. Studies on two different lamprey species have demonstrated that the incidence and rate of metamorphosis vary directly with water temperature (Manion and Stauffer 1970; Potter 1970; Purvis 1980). The evidence from these studies is not conclusive because the effect of temperature could not be clearly separated from those of other factors which also may influence metamorphosis, such as food availability and density (Morman 1987). Potter (1970) and Cole and Youson (1981) reported that light had little effect on the incidence of metamorphosis in *Mordacia mordax* or anadromous sea lamprey, respectively. The role of light stimuli is unclear because premetamorphic larvae in these experiments were subjected to continuous light and dark regimes which are atypical of their native habitats. Removal of the photosensitive pineal complex, however, prevents metamorphosis in almost all cases (Eddy 1969; Cole and Youson 1981). The objectives of our study are twofold: (1) to establish whether the size (length and weight) and CF of landlocked larval sea lamprey are useful predictors of which larvae will enter metamorphosis and (2) to assess the short-term influence of photoperiod and water temperature on the incidence of metamorphosis. Lamprey workers commonly use the term size when referring to the length of a lamprey because weight data are rarely collected. In this paper, we use size in reference to the length and weight of lamprey together.

## Materials and Methods

### Summer 1991

Larval sea lamprey were collected from the Chippewa River, Lake Huron, on May 20–23 and 27–30 and June 3–6, 1991, and brought to the Hammond Bay Biological Station where they were held in tanks at 10°C. Since past field data from the Chippewa River did not provide information on the weights of metamorphosing animals, we used values from an anadromous population in New Brunswick, Canada (Potter et al. 1978), to select larvae for our experiment in 1991. Larvae ($N = 960$) having at least one of (i) a total length ≥110 mm, (ii) a wet weight ≥2.0 g, or (iii) a CF ≥1.46 were randomly assigned to 32 experimental tanks (30.5 cm width × 60 cm length × 30 cm height) at a density of 30 animals per tank (164 larvae·m$^{-2}$). Tanks were supplied with a continuous flow of aerated, unfiltered water from Lake Huron (6–10 L·min$^{-1}$) and were placed inside cabinets with doors so that specific photoperiod regimes could be maintained throughout the experiment. Larvae were fed baker's yeast once per week and, during feeding, water to the tanks was turned off overnight, but water temperature never changed more than ±1°C in the experimental tanks. Each tank was provided with a sand substrate (5 cm) and a water depth of 13–15 cm. Light to groups of four tanks was provided by four fluorescent light tubes suspended 40 cm above the water surface. A continuous record of water temperature was obtained with thermographs in water supply tanks positioned above each group of 16 experiment tanks.

The experiment treatments consisted of two fixed water temperatures, cold (13°C) and warm (21°C), and two photoperiods, continuous darkness and an ambient 15 h light : 9 h dark regime. Mean water temperatures (SE) between June and mid-August were 13.1 (0.084)°C and 20.6 (0.073)°C. Limitations of the available facilities and equipment precluded the use of temperature and photoperiod gradients. The ambient photoperiod and warm temperature simulated the conditions to which larvae would have been exposed in the Chippewa River at the time the experiment began (data provided by R. Baker, U.S. Geological Survey, Bay City, Mich.). The colder temperature (13°C) approximated the average summer temperature of the Lake Huron supply water and was used to minimize the need for heating and cooling large volumes of water. Eight tanks were subjected to each combination of temperature and photoperiod. Since the incidence of metamorphosis is also influenced by larval density (Morman 1987), larvae from one of the eight tanks were used to replace mortalities in other tanks, eliminating possible effects of changing density. Replacement larvae were marked by a subcutaneous injection of latex dye in the caudal sinus so that they could be excluded from our data analysis.

The experiment began on June 11 and the larvae in the experimental tanks remained undisturbed until July 28 when total lengths (±1 mm), weights (±0.1 g), and stage of metamorphosis were determined. The sampling was timed so that the majority of metamorphosing animals in the warm tanks would be at stage 2 or 3 because these stages provide definitive identification of metamorphosing animals (Youson and Potter 1979). To check our initial staging of metamorphosing animals, the experiment was continued until August 10 when a final determination of the numbers and metamorphic stages was made and the weights and lengths were recorded. All stages of metamorphosis in 1991 and 1992 were determined by J.H. Youson using the descriptions in Youson and Potter (1979). A field sample of larvae and transforming animals from the Chippewa River was obtained on July 10–11, 1991, in order to compare the synchrony of metamorphosis in the field and the laboratory populations. We used the minimum observed length and weight of stage 2–3 metamorphosing animals in the laboratory at the end of July as selection criteria for the 1992 experiment.

### Summer 1992

Sea lamprey larvae were collected from the Great Chazy River, Lake Champlain region, on May 18–21, 1992. The animals were transported to Scarborough College where they were kept in tanks supplied with aerated, dechlorinated tap water at a temperature of 12–14°C, sand substrate (5 cm), and an ambient photoperiod of 15 h light : 9 h dark. We used the same fixed water temperatures as in 1991 (13 and 21°C) but photoperiod was held constant at 15 h light : 9 h dark. Instantaneous water temperature measurements were taken at least twice daily, recognizing that these data were less useful than a continuous record. Mean water temperatures (SE) during the experimental period were 13.2 (0.21)°C and 20.8 (0.12)°C. The apparatus (tank size, sediment depth) and other procedures were the same as those used at the Hammond Bay Biological Station in 1991, but only 16 experimental tanks (eight per temperature) were used and these tanks were not inside enclosed cabinets or otherwise insulated. Water temperatures in the 13°C tanks rose gradually to room temperature (19–20°C) during the time (~16 h) that water flow was stopped for weekly feeding.

Larvae that were at least 120 mm and 3.0 g ($N$ = 480) were randomly assigned to the individual tanks on June 10. The greater length and body weight of our experimental animals in the 1992 study were dictated by the results of the 1991 experiment. Lengths, weights, and stage of metamorphosis of all experimental animals were determined again on July 29 and August 12 when the experiment was formally terminated. We continued to maintain the temperature conditions into October 1992 to informally examine the effect of temperature on the timing of the different stages of metamorphosis.

### Data Analysis

The effect(s) of temperature and photoperiod on the incidence of metamorphosis among animals in the 1991 experiment, regardless of their metamorphic stage, were analyzed using two-way ANOVA. Treatment means were compared using Tukey's Studentized range test (Sokal and Rohlf 1981). The analysis of the 1992 data was similar except that we used one-way ANOVA to analyze the incidence of metamorphosis. The heterogeneity $G$-test of goodness-of-fit (Sokal and Rohlf 1981) was used to compare the predicted and observed number of metamorphosing animals in 1992. Mean values are reported with the standard error of the mean (SEM) unless indicated otherwise.

## Results

Larval sea lamprey in 1991 were 14 mm smaller and 1.3 g lighter than the larvae in 1992 as a result of the larger size selection criteria used in 1992. The mean lengths, weights, and CFs of larvae in the cold (13°C) and warm (21°C) water groups (1991 and 1992) or the dark and ambient photoperiod groups (1991 only) did not differ significantly ($P > 0.05$) at the beginning of either experiment (Fig. 1). CF in June ranged from 1.19 to 2.15 in 1991 and from 1.29 to 1.94 in 1992, but the mean CFs varied little between years compared with size.

### Incidence and Effects of Water Temperature and Photoperiod

The overall incidence of metamorphosis in 1992 (318 of 420, 76%) was higher than in 1991 (98 of 874, 11.2%), reflecting the difference in size selection criteria of the two experiments. Mortality between June and the end of July 1991 consisted of two animals in warm water (both in continuous darkness) and two animals in cold water (one in each photoperiod regime) whereas during the same period in 1992, there was no mortality. The July data in both years were consistent with the August samples, confirming our earlier staging of metamorphosing animals.

Water temperature had a strong effect on the incidence of metamorphosis ($F_{1,24} = 9.33$, $P = 0.005$) in 1991, compared with photoperiod for which no effect was detected ($F_{1,24} = 0.16$, $P = 0.70$). Significantly more animals transformed in warm water (18.9%) compared with cold water (4%), with approximately six animals metamorphosing per tank of warm water and one per tank of cold water (minimum significant difference, MSD = 2.99, $P < 0.05$) whereas three or four animals were metamorphosing in each tank when only light was considered (Table 1).

Metamorphosis of animals in the warmwater group in 1991 was synchronized with metamorphosis of animals collected from the Chippewa River in July. The majority of metamorphosing animals kept in warm water in the laboratory and those collected from the field were at stage 2 or 3 by the end of July; 36% in the warm water were at stage 2 and 56% were at stage 3. In contrast, 78% of the animals metamorphosing in cold water were at stage

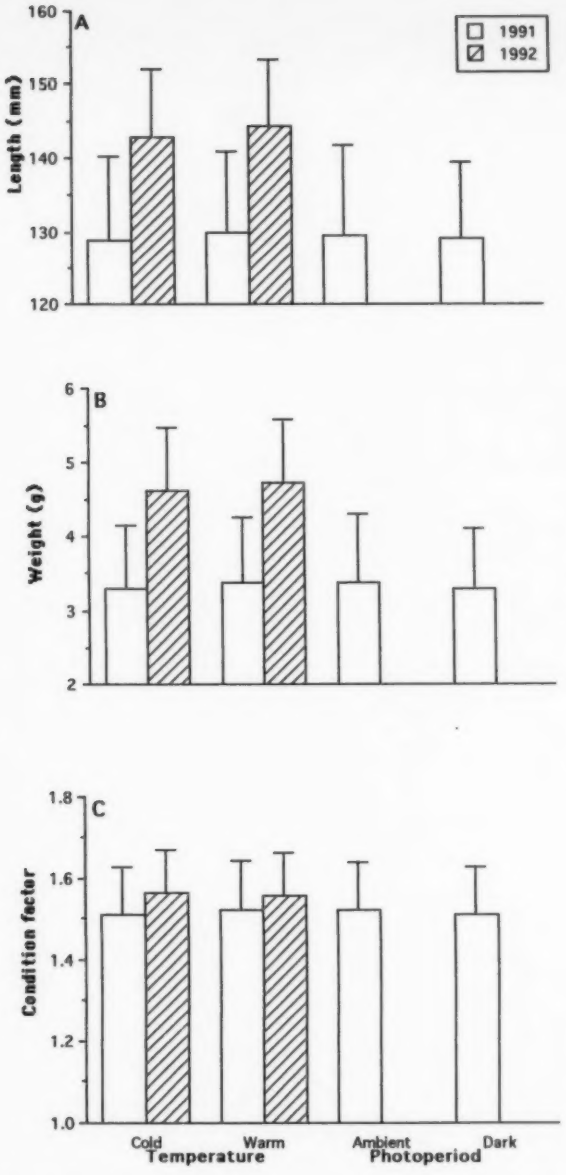



FIG. 1. Comparison of the June (A) length, (B) weight, and (C) CF of groups of larval sea lamprey from the Chippewa (1991) and the Great Chazy (1992) rivers which were subjected to two fixed temperatures (cold, 13°C and warm, 21°C). The 1991 study also compared the effects of ambient (15 h light : 9 h dark) and constant-dark photoperiods. Data are means ±1 SD.

1 on July 28, and by August their development was about 1 mo behind the development of metamorphosing animals in the warmwater group and the field population.

Water temperature also had a significant effect on the incidence of metamorphosis in 1992 ($F_{1,12} = 14.95$, $P = 0.002$), with 66% (139 of 212) of the animals in cold water metamorphosing and 84% (177 of 210) in warm water metamorphosing at the end of July. An average of 25 metamorphosing animals per tank were present in warm water and 20 per tank in cold water (Table 1).

TABLE 1. Mean (SE) number of metamorphosing sea lamprey per tank of 30 animals in 1991 and 1992. Values with an asterisk are significantly different ($P < 0.05$). The influence of photoperiod was not assessed in 1992.

| | Temperature | | Photoperiod | |
|---|---|---|---|---|
| Year | 13°C | 21°C | Dark | 15 h light : 9 h dark |
| 1991 | 1* (0.50) | 6* (1.31) | 3.8 (1.11) | 3.2 (1.21) |
| 1992 | 20* (1.16) | 25* (0.64) | | |

Photoperiod was not considered in 1992 because no effect was shown in 1991. Metamorphosing animals in the 13°C group were at stage 1 or 2 whereas 55% of metamorphosing animals in the 21°C group were at stage 3 by the end of July; thus the development of metamorphosing animals in cold water was about 2 wk behind that in warm water. The majority of animals were at stage 6 or 7 at the end of October, but few in either temperature group had left the substrate.

## Length, Weight, and CF

In both 1991 and 1992, nonmetamorphosing lamprey were approximately the same length as metamorphosing animals (Fig. 2A) but they weighed significantly less ($P < 0.05$) than metamorphosing animals up to stage 6 where weight loss in the latter was first observed (Fig. 2B). There was no discernible trend in the mean length of animals through metamorphosis, which varied between 132 and 139 mm in 1991 and 137 and 143 mm in 1992 (Fig. 2A). Similarly, in 1991 the mean weight of metamorphosing animals ranged between 3.9 and 4.4 g up to stage 6 and then declined to 3.75 g at stage 7, although the variation in the stage 7 data is greater than in earlier stages because of the small sample size ($N = 10$). In contrast, the mean weight of metamorphosing animals in 1992 declined progressively from 4.4–4.6 g in the early metamorphosing stages (1–3) to 4.0–4.1 g by stages 6 and 7.

Changes in CF during metamorphosis in both years (Fig. 2C) are similar to the pattern exhibited by weight, i.e., declining in the later stages of metamorphosis. The CFs of animals at stages 1–6 (1.62–1.69) in 1991 did not differ statistically ($P > 0.05$), but each one was significantly greater ($P < 0.05$) than the CF of nonmetamorphosing larvae (1.42) or animals at stage 7 (1.34). In 1992, the mean CF of nonmetamorphosing larvae was 1.37, which was significantly lower ($P < 0.05$) than the CF of metamorphosing animals at all stages except stage 7 (CF = 1.42); the CFs of animals in the early stages (1–3) of metamorphosis did not differ significantly among themselves ($P > 0.05$). The highest CF in 1992 was 1.73 at stage 4. The decrease in weight and CF during metamorphosis illustrates the importance of selecting length, weight, and CF criteria from data obtained from early (stages 2 and 3) metamorphosing animals.

Both larvae ($N = 43$) and metamorphosing animals ($N = 13$) collected from the Chippewa River in July 1991 were significantly larger in length and weight ($P < 0.05$) but not CF ($P > 0.05$) than animals held in the laboratory since June. By the end of July the mean (SE) length, weight, and CF of stage 2 and 3 animals in the laboratory were 134 (0.86) mm, 4.04 (0.081) g, and 1.68 (0.012) compared with 145 (1.69) mm, 5.2 (0.19) g, and 1.70 (0.029) for field animals. The smallest metamorphosing animal in the laboratory was 117 mm long, weighed 2.8 g, and had a CF of 1.50. Unmetamorphosed larvae in the laboratory had

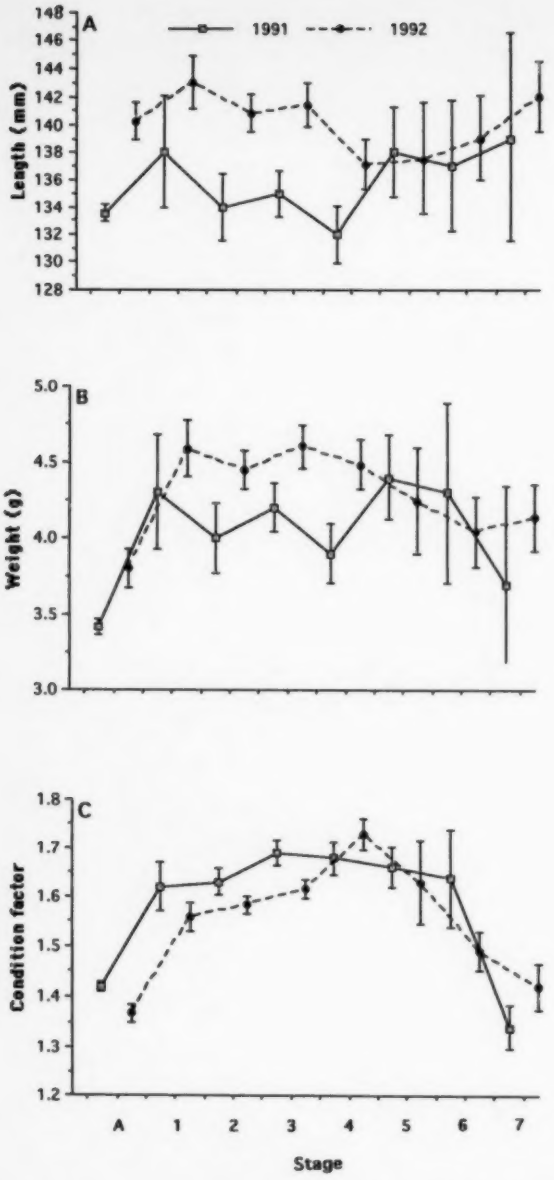



FIG. 2. Changes in the (A) length, (B) weight, and (C) CF of animals at each stage of metamorphosis (1–7) in 1991 (solid line) and 1992 (broken line). The 1992 data are offset to the right of the 1991 data for clarity. Data represent means ±95% CL. Stage A is untransformed larvae of metamorphosing size (120 mm and 3.0 g) and stages 1–7 are as described in Youson and Potter (1979). Samples from the latter stages (4–7) may include animals that were measured at early stages of metamorphosis. Sample sizes in 1991 are as follows: A, 447; 1, 23; 2, 44; 3, 93; 4, 53; 5, 27; 6, 10; 7, 10. Sample sizes in 1992 are as follows: A, 104; 1, 70; 2, 143; 3, 105; 4, 76; 5, 18; 6, 40; 7, 52.

a CF of 1.42 (0.004) which is comparable with the CF of 1.40 (0.011) of larvae that were at least 110 mm and 2.0 g in size when collected from Chippewa River in July.

The minimum and mean lengths, weights, and CFs of stage 2

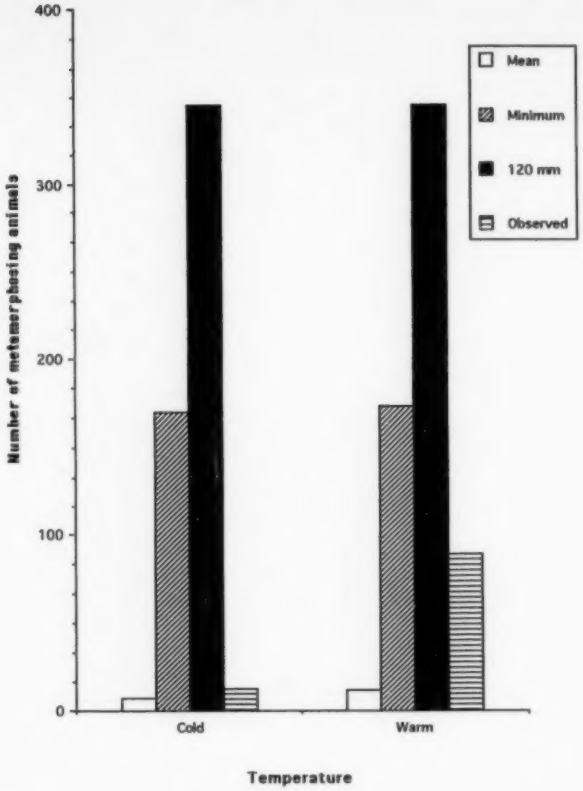



FIG. 3. Comparison of predictions of the number of animals metamorphosing from the Chippewa River with the actual number observed in late July 1992 and comparison of the effects of temperature (cold, 13°C; warm, 21°C) on the number of metamorphosing animals. Three predictors are used: (1) mean observed length (134 mm), weight (4.04 g), and CF (1.68) in late July; (2) minimum observed length (120 mm), weight (3.0 g), and CF (1.50) in late July; and (3) a length criterion of 120 mm.

and 3 animals in the July 1991 laboratory sample were used to predict from the June data the number of larvae expected to metamorphose in the warm and cold water (Fig. 3). Seven animals in cold water and 12 in warm water were predicted using the mean values (134 mm, 4.04 g, 1.68 CF) and 170 and 173, respectively, using minimum values (120 mm, 3.0 g, 1.50 CF). A length criterion of 120 mm alone predicted that 345 animals should have metamorphosed in cold water and that 345 animals also should have metamorphosed in the warm water. We observed 18 metamorphosing animals in cold water and 80 in warm water.

Based on a comparison of our predictions and observations, we used the minimum values of 120 mm and 3.0 g as criteria to select animals for the 1992 experiment. The smallest metamorphosing animal in the Great Chazy population was 119 mm long, weighed 2.7 g, and had a CF of 1.60. The mean (SE) length, weight, and CF in July were 141 (0.51) mm, 4.51 (0.048) g, and 1.60 (0.0068), respectively, whereas the observed minimums were 119 mm, 2.7 g, and 1.40. Only 16 of 316 (5%) of the metamorphosing animals had a CF below 1.50 in late July whereas in the August sample, only two animals were below this value. The means (SE) in August were 138 (0.51) mm, 4.51 (0.048) g, and

TABLE 2. Comparison of the observed and predicted number of metamorphosing sea lamprey from the Great Chazy River in 1992.

| Temperature (°C) | Total *N* | Predictor | Number metamorphosing | *G* value[a] | *P* |
|---|---|---|---|---|---|
| 13 | 212 | Observed | 139 | | |
| | | Minimum[b] | 153 | 3.26 | >0.05 |
| | | 120 mm[c] | 212 | 394.5 | <0.05 |
| 21 | 210 | Observed | 177 | | |
| | | Minimum | 156 | 12.2[d] | <0.05[d] |
| | | 120 mm | 210 | 124.5 | <0.05 |

[a]*G* value for all replicate data pooled.
[b]Presumptive metamorphosing animals had to meet all of the minimum 120 mm, 3.0 g, and a CF of 1.50 criteria.
[c]Presumptive metamorphosing animals had to meet a minimum 120-mm length criterion only.
[d]Five of seven replicate tanks are consistent with the expected frequency of metamorphosing and unmetamorphosed animals ($P > 0.05$).

1.69 (0.008) and the minimums were 117 mm, 2.5 g, and 1.40.

We tested the utility of a CF of 1.50 (combined with a minimum of 120 mm and 3.0 g) for predicting which larvae in the June 1992 experiment would enter metamorphosis in July of the same year (Table 2). Approximately 66% (139 of 212) of the animals in cold water and 84% (177 of 210) in warm water were observed metamorphosing in July whereas our selection criteria predicted from the June data that 153 (72%) larvae would metamorphose in the cold water and 156 (74%) in warm water. There tended to be fewer metamorphosing larvae than expected in replicate tanks at 13°C, but the ratio of metamorphosing to nonmetamorphosing larvae was not significantly different ($P > 0.05$) from the expected ratio of 72 (153/212) metamorphosing to 28 (59/212) nonmetamorphosing larvae. In five of the seven replicate tanks at 21°C, our observations were consistent with the expected ratio ($P > 0.05$) of 74 (156/210) metamorphosing to 26 (54/210) nonmetamorphosing larvae. However, the pooled data differed significantly ($P < 0.05$) from the expected ratio because there were more metamorphosing larvae than expected in two of the 21°C replicates.

## Discussion

Our initial size criteria (110 mm and 2.0 g) for selecting larval sea lamprey that were most likely to metamorphose were based on data from an anadromous population (Potter et al. 1978). These criteria underestimated the minimum length and weight of metamorphosing animals in the Chippewa River, Michigan, a landlocked population. The minimum observed length (117 mm) for metamorphosing animals in the Chippewa River is consistent with other evidence that 120 mm is the minimum length at which metamorphosis is initiated in landlocked sea lamprey (Potter 1980; Smith and Tibbles 1980). Weight at metamorphosis is also an important criterion, with 2.8 g being the minimum in the Chippewa River. These data support the view that metamorphosis occurs at a larger size (length and weight) in populations of landlocked sea lamprey compared with at least one population of anadromous sea lamprey (Potter and Beamish 1977; Potter et al. 1978; Potter 1980). On the other hand, the minimum and mean CF of early metamorphosing sea lamprey (stages 2 and 3) from the Chippewa River (1.50 and 1.68, respectively) differ little from minimum and mean values of 1.46 and 1.64, respectively, reported for metamorphosing animals from an anadromous population of sea lamprey (Potter et al. 1978). The value of our size (length and weight) and CF criteria for selecting presumptive metamorphosing larvae is supported by the results of the 1992 experiment on animals from the Great Chazy River, New York, in which the number of metamorphosing animals was consistent with predictions based on animals being at least 120 mm and 3.0 g in size and having a minimum CF of 1.50 in June. The minimums of 119 mm and 2.7 g were found for metamorphosing animals in this population as well as only 16 of 316 (5%) animals with a CF below 1.50. The convergence of these findings concerning CF from genetically (Wright et al. 1985) and geographically distinct populations of landlocked and anadromous sea lamprey supports the conclusion that once the minimum size (length and weight) at metamorphosis has been determined for a population, a CF of 1.50 or greater is a consistent feature of metamorphosis in this species. For assessment purposes, a presumptive metamorphosing sea lamprey in landlocked populations should be at least 120 mm long, weigh 3.0 g or more, and have a CF of 1.50 or greater.

The importance of size and physiological condition of landlocked larval sea lamprey as determinants of whether individuals are likely to enter metamorphosis is well known (Potter 1980). Physiological conditioning occurs because metamorphosis is a nontrophic interval, requiring the use of lipid reserves (Lowe et al. 1973; O'Boyle and Beamish 1977) to sustain the animal. Metamorphosing animals from the Chippewa (1991) and Great Chazy (1992) rivers had higher CFs than larvae that did not metamorphose but which were of similar lengths to metamorphosing animals. The difference in CF reflects the fact that animals in the early stages of metamorphosis (stages 1–3) were significantly heavier than unmetamorphosed larvae of the same length (Fig. 2B). The increased weight is a consequence of the immense lipid reserves stored in the tissues prior to entering metamorphosis (Lowe et al. 1973; Youson et al. 1979). Hence, CF can provide a relatively clear distinction between those larvae that will begin metamorphosis and those that will most likely wait at least another year. This difference can be used with information on size, i.e., both length and weight, to identify animals likely to enter metamorphosis more precisely than a length criterion of 120 mm alone.

The trends shown by CF during lamprey metamorphosis are related to the retention of length and a loss of weight. The absence of a reduction in length during metamorphosis is consistent with

the findings of Manion and Stauffer (1970) for another population of landlocked sea lamprey and with the findings of Potter et al. (1980) for *Geotria australis*. A massive increase in the snout and the development of associated structures during metamorphosis, showing that some protein synthesis is occurring, may compensate for small reductions in trunk length during metamorphosis (Youson 1980). The loss in weight is due to the use of body reserves, particularly lipid, for general metabolism and for various morphological and physiological changes that occur as lamprey progress through metamorphosis (Lowe et al. 1973; O'Boyle and Beamish 1977; Youson et al. 1979). Lipid levels also accumulate during the larval phase of *G. australis* (Bird and Potter 1981) and *Lampetra fluviatilis* (Moore and Potter 1976) and decline during metamorphosis in both species. This lipid loss helps to explain the marked decline in CF that has been shown to occur during metamorphosis in *G. australis* (see Potter et al. 1980). The loss in weight during lamprey metamorphosis is an important factor to consider in determining selection criteria for immediately premetamorphic lamprey. We recommend obtaining length, weight, and CF values from animals at stages 2 and 3 of metamorphosis in most populations.

In anadromous sea lamprey, CF is highest at metamorphosing stage 3 and then declines progressively such that at stage 7 and in early juveniles, it is considerably lower than in unmetamorphosed larvae (Potter et al. 1978). In contrast, CF is highest in unmetamorphosed larvae of the Southern Hemisphere lamprey *G. australis* and declines consistently throughout metamorphosis (Potter et al. 1980). The trend shown by the CF of the Great Chazy River animals during metamorphosis (Fig. 2C) is similar to the pattern exhibited by anadromous sea lamprey, although the highest CF was recorded at stage 4 in the Great Chazy population. For reasons that are not clear, the changes in CF during metamorphosis of Chippewa River animals (Fig. 2C) do not resemble the consistent trends exhibited by either anadromous sea lamprey, landlocked sea lamprey from the Great Chazy River, or *G. australis*. Age and growth rate are variable among different sea lamprey populations (Potter 1980; Beamish and Medland 1988; Holmes 1990), and these may be important factors that influence the time of initiation and the events of metamorphosis. For example, inter- and intraspecific variation in the lengths of metamorphosing lampreys implies that age as well as size is an important factor governing the onset of metamorphosis. Estimates of age derived from length–frequency data support this hypothesis, indicating that *G. australis* probably enters metamorphosis at 4.25 yr of age (Potter and Hilliard 1986) and that anadromous and landlocked sea lamprey enter metamorphosis at 6–8 yr of age (Lowe et al. 1973; Beamish and Potter 1975; O'Boyle and Beamish 1977; Potter 1980). Animals from the Chippewa River are faster growing and mature earlier than most animals in the Great Lakes region, and hence, they may enter metamorphosis at 2–3 yr of age (T.E. Medland and F.W.H. Beamish, Department of Zoology, University of Guelph, Guelph, Ont., personal communication). Statolith banding patterns in metamorphosing animals from the Great Chazy River indicate a larval life of about 5 yr for the majority of animals compared with 6 yr based on length–frequency data (Beamish and Medland 1988). Thus, variation in the age of animals entering metamorphosis in our two experimental populations may be an important factor to consider in the interpretation of differences in developmental trends.

Another important consideration in explaining variation in metamorphosis among lamprey populations are the events in the final year of larval life leading up to the initiation of the metamorphic process. The presence of the arrested growth phase during the final year of the larval period in landlocked sea lamprey may explain why CF in conjunction with size criteria is a good predictor of metamorphosis. During the arrested growth phase, there is no increase in length whereas the larvae accumulate large amounts of lipid which are used as an energy source during the nontrophic period of metamorphosis (Lowe et al. 1973; O'Boyle and Beamish 1977). This phase begins only after the animals reach a length comparable with that of metamorphosing animals. Animals about to enter the arrested growth phase were recognized in the present study as those with the minimum length 120 mm but not enough weight (less than 3.0 g) to provide the appropriate CF. In contrast, CF is not a good predictor of metamorphosis in *G. australis* (see Potter et al. 1980), probably because this species does not exhibit a conspicuous arrested growth phase in the final year of the larval period (Potter and Hilliard 1986). In the months preceding metamorphosis, *G. australis* larvae are able to utilize assimilated food to accumulate lipid reserves and increase in length at the same time, abilities that have been attributed to the size (<100 mm and 1.2 g) at which they usually enter metamorphosis (Bird and Potter 1981). CF may also be a good predictor of metamorphosis in other lamprey species that enter an arrested growth phase in the final year of the larval period including *Lampetra planeri* (Moore and Potter 1976), *Ichthyomyzon fossor* (Leach 1940), anadromous sea lamprey (Potter et al. 1978), and *M. mordax* in the Southern Hemisphere (Potter 1970). Conversely, as noted in *G. australis* (Potter et al. 1980), CF is less likely to be useful in populations of these species that do not exhibit an arrested growth phase.

The absence of a photoperiod effect on the incidence of metamorphosis in sea lamprey from the Chippewa River (Table 1) is consistent with evidence from an earlier study (Cole and Youson 1981) on metamorphosis of anadromous sea lamprey. Although photoperiod is not an important environmental cue of metamorphosis in sea lamprey, carefully controlled experiments on *L. planeri* and anadromous *P. marinus* (Eddy 1969; Cole and Youson 1981) have demonstrated that the pineal complex, which is photosensitive, is involved in regulating the onset of metamorphosis, since pinealectomy (removal of the pineal and parapineal) prevents metamorphosis in these species. The pineal complex may be a central component of the mechanism which initiates the metabolic changes that occur at the onset of metamorphosis, possibly in response to stimuli such as an increase in water temperature during the spring (Cole and Youson 1981).

Water temperature clearly influenced the incidence and rate of development of metamorphosis in sea lamprey from the Chippewa and Great Chazy rivers. More animals in water at 21°C entered metamorphosis than at 13°C in both 1991 and 1992 (Table 1), and the development of the animals in the warmer water was about 1 mo in advance of those metamorphosing in cold water. These findings are consistent with the results of earlier studies reported by Manion and Stauffer (1970), Potter (1970), Purvis (1980), and Cole and Youson (1981). However, unlike the previous studies, all factors in our study were controlled and we have demonstrated that temperature has an effect within 1 mo of the normal time when external change is first observed (i.e., stage 1). The higher incidence of metamorphosis in 1992 compared with 1991 occurred in part because we used the results of our 1991 experiment to be more selective in recognizing presumptive metamorphosing animals in 1992. However,

the two populations in our studies can be differentiated genetically (Wright et al. 1985), so the influence of genetic factors on the incidence of metamorphosis among populations of sea lamprey merits attention.

Our data on the incidence and rate of metamorphosis suggest that lower than normal temperatures may retard development during metamorphosis whereas higher temperatures may enhance the process. Similarly, Potter (1970) found that metamorphosis in *M. mordax* kept in 15°C water was delayed 4–5 wk relative to metamorphosis in animals in water at 22–25°C. The data of Purvis (1980), however, suggest that the incidence of metamorphosis exhibits a graded response to temperature as typified by the relationship between a variety of physiological and ecological rate processes (e.g., growth) and temperature in aquatic organisms (e.g., Regier et al. 1990). At the present time, we cannot accept or reject either interpretation of this relationship until we perform further studies at temperatures that are intermediate to the two fixed temperatures of the present investigation.

The rate of change of temperature in the period leading up to metamorphosis may be more important to the initiation of metamorphosis than the absolute temperature. The fixed temperatures in our experiments and those of Potter (1970) on *M. mordax* lead to the conclusion that the initiation of metamorphosis can be altered by varying environmental temperature. In addition, there is also some field evidence consistent with these experiments showing that the time of initiation of metamorphosis in *L. fluviatilis* and *L. planeri* varies in successive years according to spring temperature (Hardisty and Potter 1971b). However, in contrast with our data which showed an increased incidence of metamorphosis at the highest fixed temperature, lamprey populations in the colder regions of a species' range are often the first to enter metamorphosis (Hardisty and Potter 1971b). Clearly, there is still a need for further study on this important environmental cue of metamorphosis. For example, we have preliminary data from a long-term experiment which demonstrate that low temperatures during the winter combined with rising spring water temperatures are key factors leading to the normal initiation of metamorphosis (J.A. Holmes et al., personal observation).

The sensitivity of presumptive metamorphosing lamprey to water temperature may account for the variation in the timing of downstream migrations and in the number of individuals in these migrations from different streams in the Great Lakes region or from a particular stream for any given year. This fact has important implications for the management of sea lamprey abundance in the Great Lakes because lampricide treatments are scheduled to prevent significant numbers of animals from metamorphosing and migrating into the Great Lakes, based on knowledge about larval lengths from the previous year and knowledge of the growth rate and abundance of animals in a particular stream (Smith and Tibbles 1980).

Our laboratory experiments indicate that CF is an important and useful parameter for identifying sea lamprey in the spring that will enter metamorphosis during the summer. Our predictions of 72–74% metamorphosis in 1992 were made independently of temperature using a CF of 1.50 and are supported by the observed 66% metamorphosis in the tanks receiving cold water and to some extent by the 84% metamorphosis in the warmwater tanks. Furthermore, data from other populations of sea lamprey also appear to converge on a CF of about 1.50 as the minimum value of an animal in the earliest stages of metamorphosis. The differences in the observed and predicted incidences of metamorphosis are a consequence of the influence of temperature. A minimum CF of 1.50 in combination with minimum size criteria of 120 mm and 3.0 g provides a more accurate tool for identifying premetamorphic animals than a 120-mm length criterion alone.

## Acknowledgements

We thank the technical staff at the Hammond Bay Biological Station (HBBS) for their help during various aspects of the study in 1991 and the staffs of the Ludington and Marquette biological stations who aided in the collection of animals in 1991 and 1992. Bill Swink and Roger Bergstedt of the HBBS provided useful comments during the course of our study. Geoffrey Locke provided careful attention to the design and building of the experimental system at the HBBS. This study was primarily supported by contract award to J.H.Y., J.G.S., S.A.S., and F.W.H.B. by the Great Lakes Fishery Commission and was supplemented by funds from a Natural Sciences and Engineering Research Council of Canada grant awarded to J.H.Y.

## References

APPLEGATE, V.C. 1950. Natural history of the sea lamprey, *Petromyzon marinus*, in Michigan. U.S. Fish Wildl. Serv. Fish. 55: 1–237.

BEAMISH, F.W.H., AND T.E. MEDLAND. 1988. Age determination for lampreys. Trans. Am. Fish. Soc. 117: 63–71.

BEAMISH, F.W.H., AND I.C. POTTER. 1975. The biology of the anadromous sea lamprey (*Petromyzon marinus*) in New Brunswick. J. Zool. (Lond.) 177: 57–72.

BIRD, D.J., AND I.C. POTTER. 1981. Proximate body composition of the larval, metamorphosing and downstream migrant stages in the life cycle of the Southern Hemisphere lamprey, *Geotria australis*. Environ. Biol. Fishes 6: 285–297.

COLE, W.C., AND J.H. YOUSON. 1981. The effect of pinealectomy, continuous light, and continuous darkness on metamorphosis of anadromous sea lampreys, *Petromyzon marinus* L. J. Exp. Zool. 218: 397–404.

EDDY, J.M.P. 1969. Metamorphosis and the pineal complex in the brook lamprey, *Lampetra planeri*. J. Endocrinol. 44: 451–452.

HARDISTY, M.W., AND I.C. POTTER. 1971a. The behaviour, ecology and growth of larval lampreys, p. 88–125. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London, U.K.

HARDISTY, M.W., AND I.C. POTTER. 1971b. The general biology of adult lampreys, p. 127–206. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 1. Academic Press, London, U.K.

HOLMES, J.A. 1990. Sea lamprey as an early responder to climate change in the Great Lakes basin. Trans. Am. Fish. Soc. 119: 292–300.

LEACH, W.J. 1940. Occurrence and life history of the northern brook lamprey, *Ichthyomyzon fossor*, in Indiana. Copeia 1940: 21–34.

LOWE, D.R., F.W.H. BEAMISH, AND I.C. POTTER. 1973. Changes in the proximate body composition of *Petromyzon marinus* (L.) during larval life and metamorphosis. J. Fish Biol. 5: 673–682.

MANION, P.J., AND T.M. STAUFFER. 1970. Metamorphosis of the landlocked sea lamprey, *Petromyzon marinus*. J. Fish. Res. Board Can. 27: 1735–1746.

MOORE, J.W., AND I.C. POTTER. 1976. Aspects of feeding and lipid deposition and utilization in the lampreys, *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch). J. Anim. Ecol. 45: 699–712.

MORMAN, R.H. 1987. Relationship of density to the growth and metamorphosis of caged larval sea lampreys, *Petromyzon marinus* Linnaeus, in Michigan streams. J. Fish. Biol. 30: 173–181.

O'BOYLE, R.N., AND F.W.H. BEAMISH. 1977. Growth and intermediary metabolism of larval and metamorphosing stages of the landlocked sea lamprey, *Petromyzon marinus* L. Environ. Biol. Fishes 2: 103–120.

POTTER, I.C. 1970. The life cycles and ecology of Australian lampreys of the genus *Mordacia*. J. Zool. (Lond.) 161: 487–511.

POTTER, I.C. 1980. Ecology of larval and metamorphosing lampreys. Can. J. Fish. Aquat. Sci. 37: 1641–1657.

POTTER, I.C., AND F.W.H. BEAMISH. 1977. The freshwater biology of adult anadromous sea lampreys *Petromyzon marinus*. J. Zool. (Lond.) 181: 113–130.

POTTER, I.C., AND R.W. HILLIARD. 1986. Growth and the average duration of larval life in the Southern Hemisphere lamprey, *Geotria australis* Gray. Experientia 42: 1170–1173.

POTTER, I.C., R.W. HILLIARD, AND D.J. BIRD. 1980. Metamorphosis in the

Southern Hemisphere lamprey, *Geotria australis*. J. Zool. (Lond.) 190: 405–430.

POTTER, I.C., R.W. HILLIARD, AND D.J. BIRD. 1982. Stages in metamorphosis, p. 137–164. *In* M.W. Hardisty and I.C. Potter [ed.] The biology of lampreys. Vol. 4B. Academic Press, London, U.K.

POTTER, I.C., G.M. WRIGHT, AND J.H. YOUSON. 1978. Metamorphosis in the anadromous sea lamprey, *Petromyzon marinus* L. Can. J. Zool. 56: 561–570.

PURVIS, H.A. 1980. Effects of temperature on metamorphosis and the age and length at metamorphosis in sea lamprey (*Petromyzon marinus*) in the Great Lakes. Can. J. Fish. Aquat. Sci. 37: 1827–1834.

REGIER, H.A., J.A. HOLMES, AND D. PAULY. 1990. Influence of temperature changes on aquatic ecosystems: an interpretation of empirical data. Trans. Am. Fish. Soc. 119: 374–389.

RICKER, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191.

SMITH, B.R., AND J.J. TIBBLES. 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan and Superior: history of invasion and control, 1936–78. Can. J. Fish. Aquat. Sci. 37: 1780–1801.

SOKAL, R.R., AND F.J. ROHLF. 1981. Biometry. 2nd ed. W.H. Freeman and Co., New York, N.Y. xviii + 859 p.

WRIGHT, J., C.C. KRUEGER, P.F. BRUSSARD, AND M.C. HALL. 1985. Sea lamprey (*Petromyzon marinus*) populations in northeastern North America: genetic differentiation and affinities. Can. J. Fish. Aquat. Sci. 42: 776–784.

YOUSON, J.H. 1980. Morphology and physiology of lamprey metamorphosis. Can. J. Fish. Aquat. Sci. 37: 1687–1710.

YOUSON, J.H. 1988. First metamorphosis, p. 135–196. *In* W.S. Hoar and D.J. Randall [ed.] Fish physiology. Vol. XIB. Academic Press, New York, N.Y.

YOUSON, J.H., J. LEE, AND I.C. POTTER. 1979. The distribution of fat in larval, metamorphosing, and young adult anadromous sea lampreys, *Petromyzon marinus* L. Can. J. Zool. 57: 237–246.

YOUSON, J.H., AND I.C. POTTER. 1979. A description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus* L. Can. J. Zool. 57: 1808–1817.

YOUSON, J.H., G.M. WRIGHT, AND E.C. OOI. 1977. The timing of changes in several internal organs during metamorphosis of anadromous larval lamprey, *Petromyzon marinus*. Can. J. Zool. 55: 469–473.

# Geographic Variation in the Spawning of Atlantic Cod, *Gadus morhua*, in the Northwest Atlantic

Jeffrey A. Hutchings, Ransom A. Myers, and George R. Lilly

*Department of Fisheries and Oceans, Science Branch, P.O. Box 5661, St. John's NF A1C 5X1 Canada*

Hutchings, J.A., R.A. Myers, and G.R. Lilly. 1993. Geographic variation in the spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 50: 2457–2467.

We analyzed 47 yr (1946–92) of research trawl data and 5 yr (1964–68) of research gillnet data to identify spawning locations of Atlantic cod, *Gadus morhua*, in the Newfoundland–Labrador region. Offshore, spawners are common on the continental shelf but generally rare on the slope. Relative abundance of spawning individuals on the shelf is comparably high off northeast Newfoundland, within 100 km of the Newfoundland coast from Cape Freels to Cape Race, on central Grand Bank, and on St. Pierre Bank. Slope spawning is largely restricted to the eastern slope of Hamilton Bank, a small section of northern Grand Bank, and Flemish Cap. Inshore spawning is evident in southeastern Labrador and southeastern Newfoundland, particularly in St. Mary's, Placentia, Trinity, and Bonavista bays. Trajectories of satellite-tracked drifter buoys indicate that it is highly improbable that eggs spawned on the slope of Grand Bank and much of the northeastern Newfoundland slope will be transported into shelf and coastal waters. We conclude that cod spawn in areas in which their eggs and larvae are likely to be retained and that inshore spawning populations may provide a considerably larger contribution to cod recruitment in coastal Newfoundland than has previously been believed.

Nous avons analysé les données de recherche recueillies pendant 47 a (1946–1992) à l'aide de chaluts et celles obtenues sur 5 a (1964–1968) avec des filets maillants dans le but de déterminer l'emplacement des frayères de la morue franche, *Gadus morhua*, dans la région de Terre-Neuve et du Labrador. Au large, on trouve beaucoup de géniteurs sur le plateau continental mais généralement peu sur la pente. On observe à peu près la même abondance relative de géniteurs au large du nord-est de Terre-Neuve, à moins de 100 km de la côte de Terre-Neuve entre Cape Freels et Cape Race, au centre du Grand Banc et sur le Banc de Saint-Pierre. Le frai sur le talus continental se limite en gros à la pente est du Banc Hamilton, une petite zone de la partie nord du Grand Banc, et au Bonnet Flamand. La reproduction côtière se fait au sud-est du Labrador et de Terre-Neuve, notamment dans les baies Sainte-Marie, de Plaisance, de la Trinité et de Bonavista. D'après les trajectoires des balises itinérantes suivies par satellite, il est très peu probable que les oeufs pondus sur la pente du Grand Banc et sur la majeure partie de la pente du nord-est de Terre-Neuve soient transportés jusque dans les eaux du plateau et de la côte. On en conclut que la morue fraie dans des zones où les oeufs et les larves ont des chances de rester et que les populations qui fraient dans les eaux côtières contribuent sans doute beaucoup plus qu'on ne le pensait au recrutement de la morue aux abords de Terre-Neuve.

*Received March 26, 1993*
*Accepted September 9, 1993*
*(JB863)*

*Reçu le 26 mars 1993*
*Accepé le 9 septembre 1993*

The successful management of an exploited species or population requires reliable knowledge of the environment in which individuals reproduce. In the absence of such basic information, the relative importance of biological and physical environmental factors on variability in reproductive success and survival cannot be fully assessed. In the Northwest Atlantic off Newfoundland and Labrador, the Atlantic cod, *Gadus morhua*, is a species of great commercial importance for which few data on spawning location exist. Based largely upon the ichthyoplankton surveys described by Serebryakov (1965, 1967) and two research cruises conducted in the early 1960s by Templeman (1965), cod have typically been described as spawning primarily over the continental slope with the greatest concentration of spawning individuals located off southeastern Labrador (Serebryakov 1965, 1967; Templeman and May 1965; Fitzpatrick and Miller 1979; Templeman 1981). The spawning locations identified by Fitzpatrick and Miller (1979) (Fig. 1), based almost entirely upon the aforementioned work, are currently accepted as the major spawning locations of cod in the Newfoundland–Labrador region (e.g., Templeman 1981; Helbig et al. 1992; de Young and Rose 1993). However, as noted by Helbig et al. (1992), eggs and larvae produced over most of the purported spawning areas would be transported away from the continental shelf and coastal bays and would likely be lost to the population(s).

Our first objective is to address the inconsistency that underlies the reported spawning areas and the predicted trajectories of egg and larval transport. We combine offshore research data from 1946 to 1992 to determine the geographical distribution of spawning individuals throughout the continental shelf and slope waters. Secondly, by incorporating variability in sampling effort, we calculate indices that describe the relative abundance of spawning individuals in the Newfoundland–Labrador region. Our third objective is to collate all data on spawning cod in coastal areas in order to assess the prevalence of inshore spawning. Finally, we include a review of extant research spawning

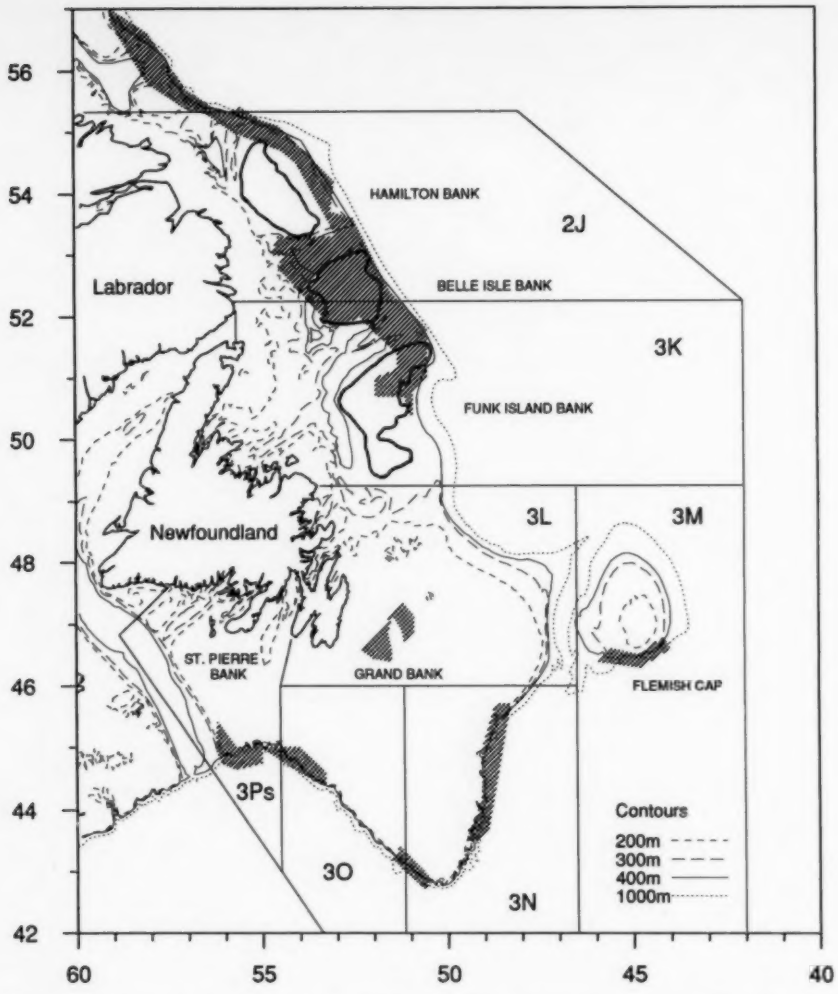



FIG. 1. Spawning locations (hatched areas) of Atlantic cod identified by Fitzpatrick and Miller (1979) off Newfoundland and Labrador. Hamilton, Belle Isle, and Funk Island banks are delineated along their respective 200-m (Hamilton Bank) and 300-m depth contours. Number and letter codes indicate NAFO Divisions.

records of cod off Newfoundland and Labrador in order to evaluate our results within a historical and biological context.

## Materials and Methods

### Offshore Region

Offshore spawning locations were determined from research trawl data collected from 1946 to 1992 over the continental shelf and slope off Newfoundland and Labrador. The geographical area extended from Hamilton, Belle Isle, and Funk Island banks (NAFO Divisions 2J and 3K) in the north to Grand Bank (3LNO), Flemish Cap (3M), and St. Pierre Bank (3Ps) in the south (Fig. 1). Our definitions of the continental shelf and slope in the Newfoundland–Labrador region conform to those used by physical oceanographers (e.g., Helbig et al. 1992). The northeast Newfoundland shelf (NAFO Divisions 2J and 3K) is defined by the shelf break between 300–400 and 1000 m although it includes deep (>200 m) bays and a deeper (up to 500 m) basin. Eastward and southward, the shelf broadens somewhat to form Grand Bank and St. Pierre Bank and the slope begins at the 200-m isobath. Trawl data were available for most years from 1962 to 1992 in all regions except Flemish Cap where annual data were limited largely to 1977–85 (Table 1). From 1946 to 1961, data were available for most years for the Grand and St. Pierre banks only (prior to 1962, 2J was sampled only once during the spawning period, while 3K was not sampled).

Reproductive status of female and male cod was assessed following the criteria defined by Templeman et al. (1978). Females were deemed to be in spawning condition if clear eggs were present in the ovary (clarity being indicative of hydration). Given that eggs will normally be released within 3 d of hydration (Kjesbu et al. 1990), the collection locations of females containing hydrated eggs should closely approximate spawning locations. Females were deemed to be spent if their ovaries were

TABLE 1. Research vessels and years in which otter trawls were used, by NAFO Region, during the spawning periods (see text for months) of Atlantic cod off Newfoundland and Labrador:

| NAFO Region | Years sampled | Research vessel |
|---|---|---|
| 2J (HamiltonBank) | 1958 | *Investigator II* |
| | 1962–64, 1966, 1969–71, 1973–74 | *A.T. Cameron* |
| | 1978–79, 1981–83, 1986–92 | *Gadus Atlantica* |
| | 1991 | *Alfred Needler* |
| | 1992 | *Northern Kingfisher* |
| 3K (northeastern Newfoundland) | 1963–64, 1969–71, 1974 | *A.T. Cameron* |
| | 1978–83, 1985–91 | *Gadus Atlantica* |
| | 1992 | *Northern Kingfisher* |
| 3L (including northern Grand Bank) | 1946–48, 1950–51, 1953 | *Investigator II* |
| | 1959, 1961, 1963–78, 1980–82 | *A.T. Cameron* |
| | 1979–80, 1984–90 | *Gadus Atlantica* |
| | 1983–92 | *Wilfred Templeman* |
| | 1984 | *Alfred Needler* |
| 3M (Flemish Cap) | 1961, 1964, 1968, 1977 | *A.T. Cameron* |
| | 1978–85 | *Gadus Atlantica* |
| 3N (southeastern Grand Bank) | 1946–48, 1950–53, 1956–59 | *Investigator II* |
| | 1959–62, 1964–82 | *A.T. Cameron* |
| | 1984–85 | *Alfred Needler* |
| | 1985–92 | *Gadus Atlantica* |
| 3O (southwestern Grand Bank) | 1946–48, 1951–52, 1955–59 | *Investigator II* |
| | 1959–62, 1964–66, 1968, 1971, 1973, 1975–82 | *A.T. Cameron* |
| | 1984–85 | *Alfred Needler* |
| | 1985–91 | *Gadus Atlantica* |
| 3Ps (St. Pierre Bank) | 1947–48, 1950–51, 1953–54, 1956–60, 1963, 1965 | *Investigator II* |
| | 1960, 1962, 1964, 1967–70, 1972–82 | *A.T. Cameron* |
| | 1984–85 | *Alfred Needler* |
| | 1985–87 | *Wilfred Templeman* |
| | 1986 | *Gadus Atlantica* |

whitish grey or bluish grey, slack, and often wrinkled; residual eggs may have been present. Males were defined as spawning individuals if their testes and vasa deferentia were white and not depleted of milt. Males were deemed to be spent if their testes were grey or pink and if milt was not evident in the outer edges at the time when all females were spent.

To identify spawning locations of cod, we initially plotted the locations in which research gear had sampled at least one spawning female or male. We then restricted our analysis to research trawl data to quantify the relative abundance of spawning individuals offshore. This analysis incorporated the geographical bias in spawning concentrations induced by variation in sampling effort. Effort was defined as the number of research trawls (each of approximately 30-min duration) conducted in a given unit area during the 4 months in which most spawning occurs. These regional time periods, as determined from Myers et al. (1993), were February–May for 3M, March–June for 2J, 3K, 3N, 3O, and 3Ps, and April–July for 3L. Generally, a subset of all cod captured in a given trawl were sampled for state of maturity. For each of these subsamples, we calculated the proportion of fish greater than the mean length of maturity (calculated separately for each NAFO Division) that were in a spawning state. We then calculated the number of spawning fish per trawl by multiplying the actual number of fish greater than the mean length at maturity by the incidence of the spawning state among fish of the same size in the subsample. If fewer than five individuals greater than the mean length at maturity were examined for spawning condition, the trawl was excluded from the analysis. We report the median number of spawning fish per trawl to reduce the effect of rare trawls in which very large numbers of cod were caught. The number of spawning fish per trawl will be related to population density (e.g., if population density is low in one region, the maximum number of spawners per trawl will be less than observed elsewhere). Trawls that were damaged during operation (i.e., deemed to have adversely affected the catch in some manner) or fished at depths below which cod are generally not found (>500 m; e.g., Baird et al. 1992) were excluded from the analysis. A total of 3181 trawls met our criteria and were included in the analysis. The size of the unit areas depended upon the region and was designed to maximize the number of trawls per unit area but minimize the size of each area. Sampling effort was higher in the south primarily because of the difficulty that ice conditions impose on sampling of spawning cod in the north. Thus, unit areas were relatively small but numerous in the south, being 0.5° latitude × 0.5° longitude on Grand Bank ($n$ = 77, 34, and 32 unit areas in 3L, 3N, and 3O, respectively), St. Pierre Bank ($n$ = 30 in 3Ps), and Flemish Cap ($n$ = 18 in 3M) (Fig. 2). Unit areas were larger in the north (1° latitude × 1° longitude) and fewer in number ($n$ = 14 and 13 in 3K and 2J, respectively). Unit areas in which only one or two trawls were sampled were excluded from the analysis (Fig. 2). This criterion removed 6% ($n$ = 13) of the unit areas.

Trawl data were obtained from six research vessels. Of these,

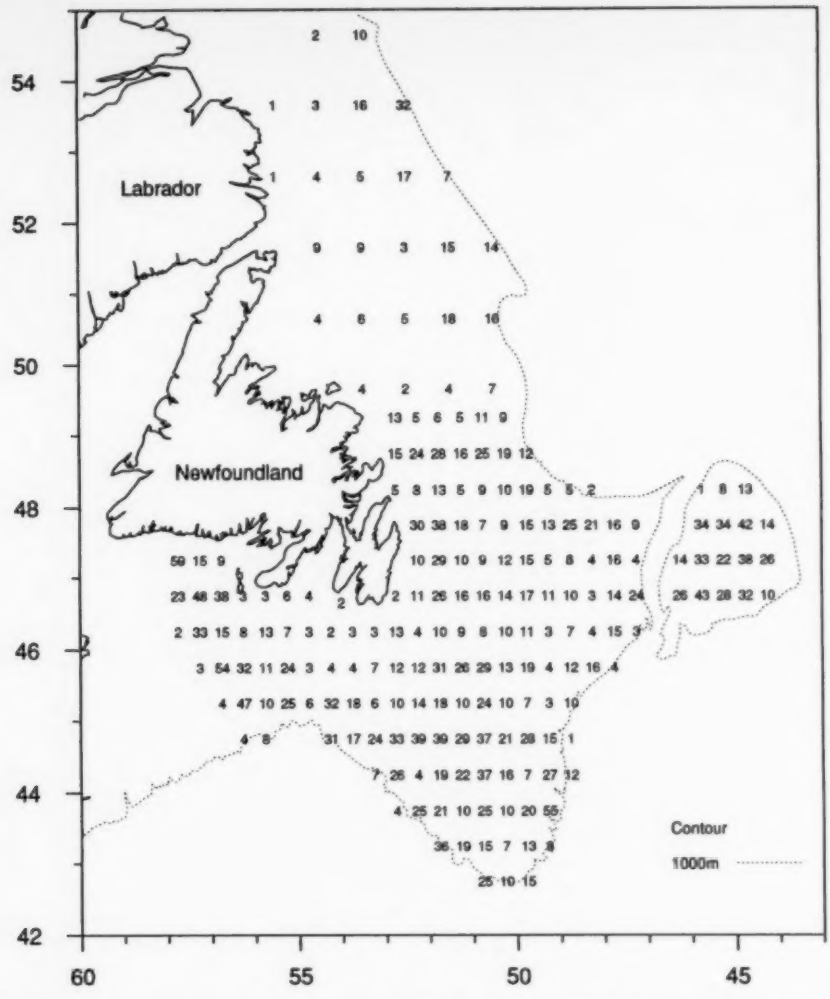



FIG. 2. Number of research otter trawls conducted from 1946 to 1992 during potential Atlantic cod spawning periods within unit areas of the continental shelf and slope off Newfoundland and Labrador (the sizes of the unit areas and the potential spawning periods specific to different regions are described in the text). Only those trawls in which cod were sampled for state of maturity are included here.

the *A.T. Cameron* and *Wilfred Templeman* have been shown to be equally efficient in catching cod (Gavaris and Brodie 1984). The *Alfred Needler* can be included with these, as it is the sister ship of the *Wilfred Templeman*. The trawls fished by the *Gadus Atlantica* are the same as those fished by the *Wilfred Templeman* and the *Alfred Needler*. The remaining ships, the *Investigator II* and *Northern Kingfisher*, contributed 212 and 46 trawls, respectively; their total contribution represents only 3% of the total number of trawls in the analysis. We therefore assumed that sampling efficiency was similar among research vessels. We pooled all data because our intent was to identify spawning sites that either have been or are presently being utilized by cod. In addition, there is no evidence that either the months in which trawls were conducted or the average number of spawning females per trawl has changed significantly through time. Thus, it is reasonable to assume that our descriptions represent "average" depictions of spatial variation in cod spawning for the time period examined.

The relative abundance of spawning cod on the shelf versus the slope was compared with a one-way ANOVA. The probability of obtaining the observed $F$-statistic in each analysis was assessed from 1000 data randomizations (cf. Manly 1991).

### Inshore Region

Data on inshore spawning were obtained primarily from research trawl and gillnet surveys. From 1964 to 1966, the Fisheries Research Board of Canada conducted experimental gillnet surveys at two stations in St. Mary's Bay (Colinet Island and Broad Cove) and two in Placentia Bay (Merasheen and Woody Point). Additional gillnet sampling was conducted in 1967 and 1968 in Trinity Bay (Horse Chops and Tickle Harbour Point) and Bonavista Bay (Cabot Island and Little Denier ). The details of these surveys are available in cruise reports of the *M.V. Marinus* (reports 1964(1), 1965(1), 1966(1), 1967(1), and

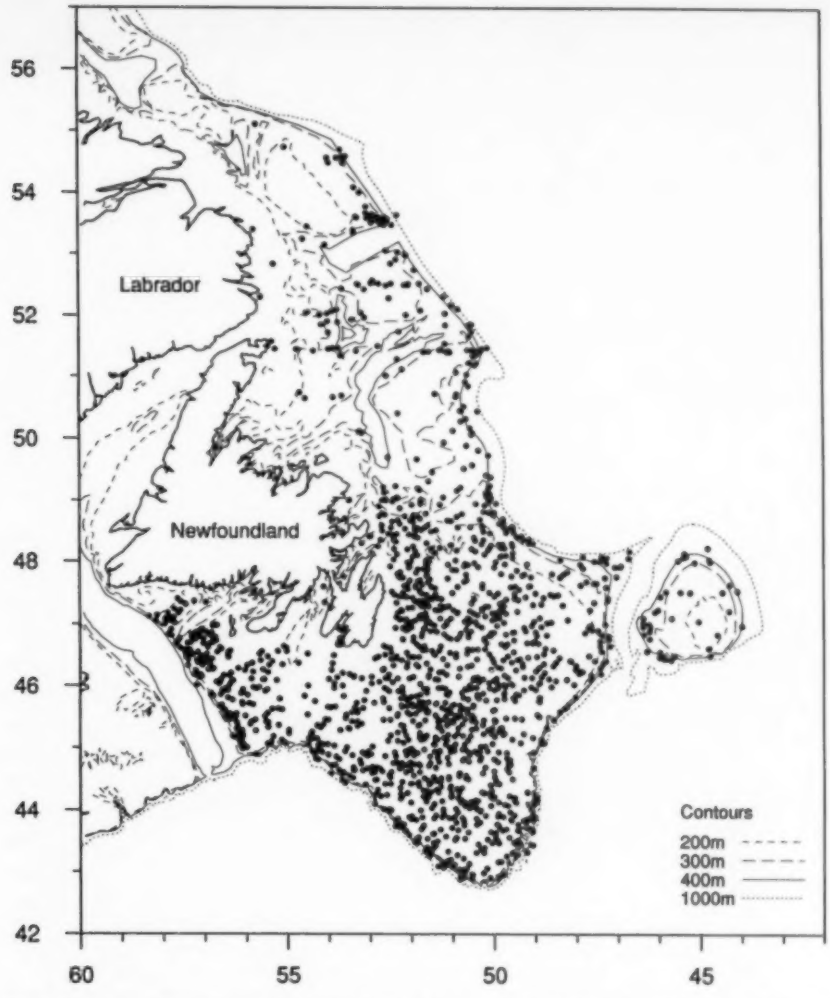



FIG. 3. Locations of Atlantic cod (male and female ) in spawning condition sampled by research trawls, gill nets, and jiggers from 1946 to 1992 off Newfoundland and Labrador.

1968(1 and 2)). The gill nets measured 91.4 m in length and contained mesh sizes of 6 in. (Trinity and Bonavista bays), 6.5 in. (St. Mary's and Placentia bays), or 7 in. (all bays). An index of abundance was calculated for each station by dividing the total number of spawning males and females caught by the total number of gill nets fished. The relative abundance of spawning cod among bays was compared with a one-way ANOVA. Statistical significance was evaluated by the randomization method, using 1000 data randomizations (Manly 1991). Maturity data on inshore cod were also obtained from samples collected by commercial gill nets off Cape Bonavista in 1983 and 1984 and by research jiggers off Labrador.

## Results

### Offshore Spawning

Spawning cod have been captured throughout the continental shelf and slope of Newfoundland and Labrador (Fig. 3). Shelf spawning was evident on all banks and on Flemish Cap. The lower frequency of collections at shelf locations in 2J and 3K is a reflection of the difficulty that ice cover imposes on sampling during the spawning period. Spawning individuals were captured on the slopes of all banks, including Flemish Cap.

Spawning was concentrated primarily in shelf areas (Fig. 4). Labrador shelf spawning was evident on southeastern Hamilton Bank and Belle Isle Bank, while spawning occurred throughout the northeastern Newfoundland shelf. Off southeastern Newfoundland, spawner abundance was highest within 100 km of the coast and on central Grand Bank. Throughout Grand and St. Pierre banks, spawning fish were encountered more frequently on the shelf than on the slope. Only off Hamilton Bank (2J) were reproductive individuals more abundant on the slope than they were further inshore (although this may reflect spatial bias in sampling). The relative abundance of spawning cod in unit areas located on the shelf (mean ± SD: 5.16 ± 5.91, $n = 135$) was more than double that for unit areas located on the slope

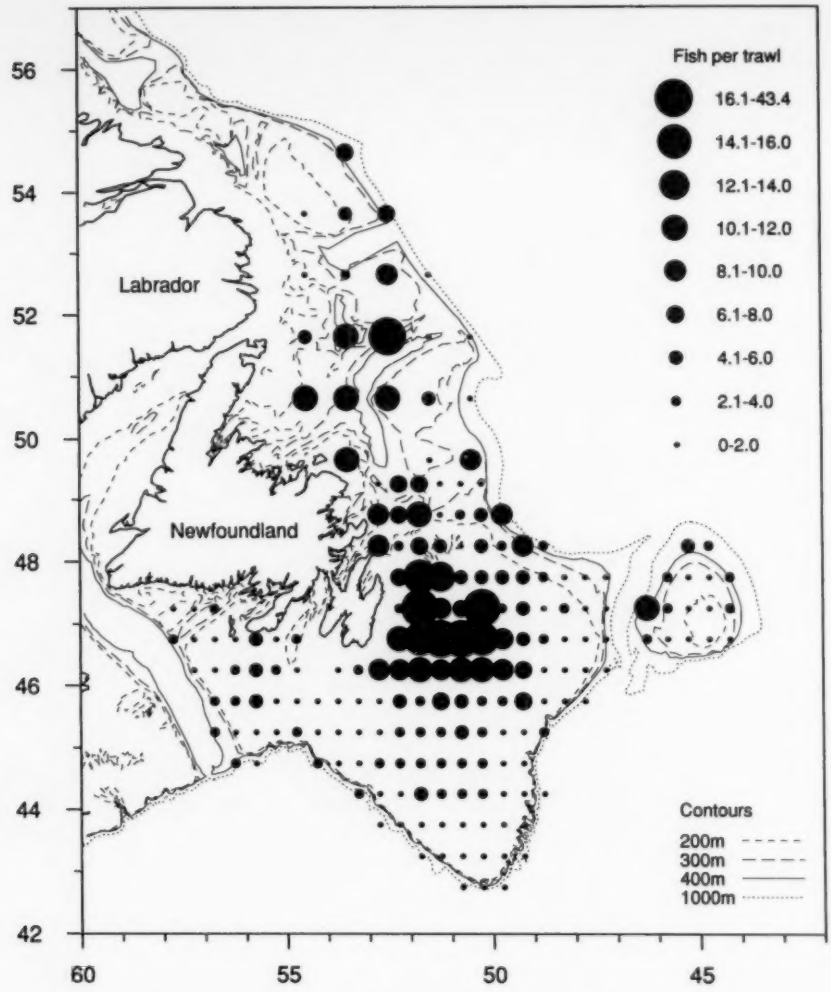



FIG. 4. Relative abundance of spawning Atlantic cod (sexes combined) on the continental shelf and slope off Newfoundland and Labrador. The circles represent the median number of spawning fish caught per trawl in each unit area during potential spawning periods (see text) from 1946 to 1992.

(2.38 ± 2.64, $n$ = 61); the difference was highly significant ($F_{[1,194]} = 12.32, p < 0.001$).

## Inshore Spawning

Spawning cod have been captured throughout the coastal regions of southeastern Newfoundland and in southeastern Labrador and northern Newfoundland (Fig. 5). The experimental gillnet surveys in St. Mary's, Placentia, Trinity, and Bonavista bays yielded an abundance of spawning cod in both the inner and outer reaches of each bay (Table 2). The percentage of catches composed of spawning individuals was higher in the May–June sampling period than it was during the April–May period in all but St. Mary's Bay (in which catch rates were similar between periods). The relative abundance of spawning cod, measured as the number of fish per gill net, did not differ significantly among bays (St. Mary's Bay: 16.1 ± 25.5, Placentia Bay: 6.7 ± 3.0, Trinity Bay: 4.0 ± 2.3, Bonavista Bay: 8.2 ± 2.3; $F_{[3,16]} = 0.77$, $p = 0.531$). The magnitude of the standard deviation for St. Mary's Bay is inflated due to the extremely high value of 67.5 fish per net in 1964. When this value was excluded, the abundance for St. Mary's Bay decreased to 5.8 ± 4.3 (the abundances among bays remained non-significant, $F_{[3,15]} = 1.60$, $p = 0.236$).

Further evidence of inshore spawning was provided by the sample of mature individuals collected by commercial gill nets fished immediately off Cape Bonavista in May–July 1983 and 1984. Although gonads were not examined to determine the state of maturity, the spawning condition of these fish can be assessed with an index that reflects the proportional allocation of body mass to gonads versus soma. In the absence of data on body weight, we divided gonad weight by (body length)$^3$ and compared the value of this gonadal index for the fish sampled by the commercial nets with the mean value for fish known to be in spawning condition in 3L (gonadal index in 3L: 0.143 ± 0.060, $n$ = 275, including all research data from 1946 to 1992). Of the

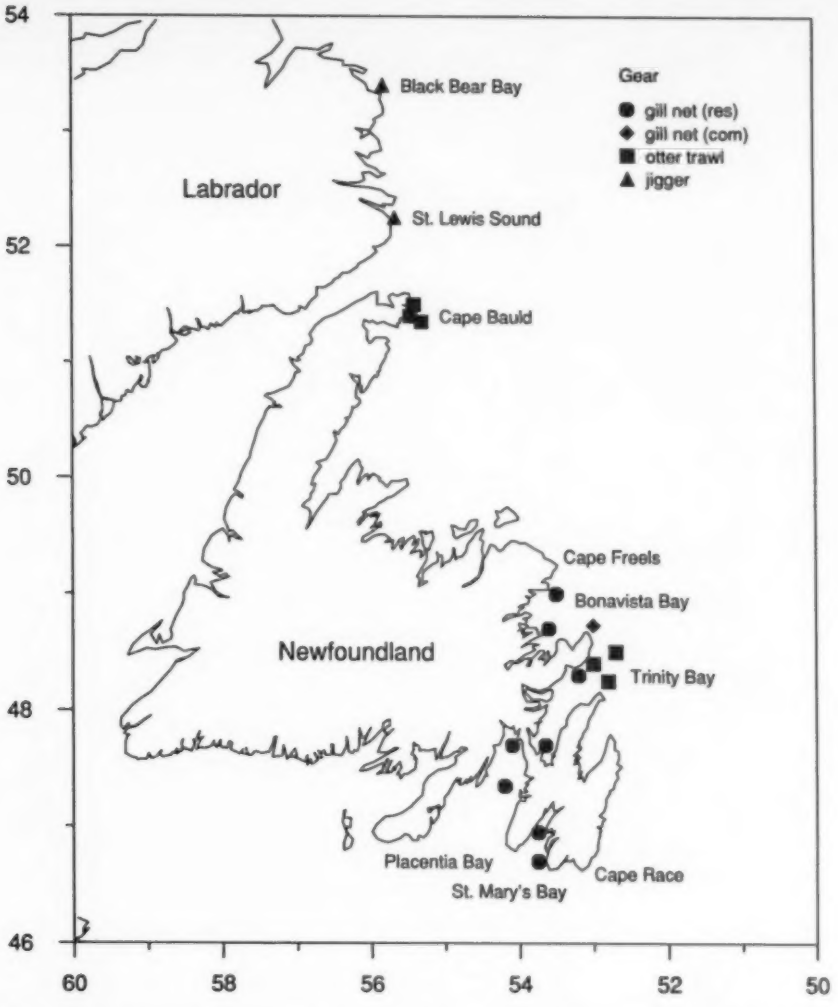



FIG. 5. Inshore locations of spawning Atlantic cod (sexes combined) sampled by gill nets (primarily research), research otter trawls, and research jiggers in coastal Newfoundland from 1946 to 1992.

315 female cod sampled in May–July 1983 and 1984, the gonadal index of 21% of the individuals encompassed 1 SD of the mean gonadal index of spawning cod in 3L, providing evidence of spawning immediately off Cape Bonavista.

Inshore cod in spawning condition have also been captured by research otter trawls and jiggers. Trawl samples containing spawning cod have been conducted in the outer reaches of Trinity Bay and close to shore near Cape Bauld (Fig. 5). Spawning cod have also been collected in Black Bear Bay and St. Lewis Sound, southeastern Labrador, by research jiggers.

## Discussion

### Cod Spawning Locations

Our data indicate that Atlantic cod spawn throughout the continental shelf and slope waters of Newfoundland and Labrador. However, contrary to previous views (Serebryakov 1965, 1967; Templeman 1965, 1981; Fitzpatrick and Miller 1979), research trawl samples of mature individuals indicate that Newfoundland cod, consistent with Barents Sea stocks (Bergstad et al. 1987), spawn primarily on the shelf. The relative abundance of spawning individuals on the shelf is comparably high: (i) off northeast Newfoundland, (ii) within 100 km of the Newfoundland coast from Cape Freels to Cape Race, (iii) on central Grand Bank, and (iv) on St. Pierre Bank. Slope spawning is largely restricted to the eastern slope of Hamilton Bank, a small section of northern Grand Bank, and Flemish Cap. Spawning is also evident in inshore waters with high concentrations recorded in St. Mary's, Placentia, Trinity, and Bonavista bays in southeastern Newfoundland. Two factors may contribute to the relatively low numbers of spawning fish per trawl in some areas. Experimental observations (Brawn 1961) indicate that cod undergo vertical movements in the water column during spawning. Bottom trawls may not efficiently capture spawning cod. Secondly, the numbers of females classified as in spawning condition, i.e., as containing hydrated eggs, may have been underestimated. Kjesbu et al. (1990) have reported that eggs in

TABLE 2. Numbers of male and female Atlantic cod in spawning condition sampled from experimental gill nets in inshore Newfoundland (nd = no data).

| Location | Sampling period | Sample size | | Percent in spawning condition | | Spawning fish per net |
|---|---|---|---|---|---|---|
| | | Male | Female | Male | Female | |
| Colinet Island, | May–June 1964 | 295 | 120 | nd | >50 | 13.4 |
| St. Mary's Bay | May–June 1965 | 330 | 98 | 70 | 57 | 4.5 |
| | April–May 1966 | 247 | 118 | 11 | 65 | 3.1 |
| Broad Cove, | May–June 1964 | 337 | 68 | nd | 34–91 | 67.5 |
| St. Mary's Bay | May–June 1965 | 148 | 35 | 82 | 73 | 3.6 |
| | April–May 1966 | 297 | 94 | 85 | 81 | 4.6 |
| Merasheen, | May–June 1964 | 131 | 71 | >50 | >50 | 7.8 |
| Placentia Bay | April–May 1965 | 220 | 138 | 26 | 20 | 4.3 |
| | April–May 1966 | 277 | 178 | 16 | 22 | 3.9 |
| Woody Island, | May– June 1964 | 39 | 31 | >50 | >50 | 1.8 |
| Placentia Bay | April–May 1965 | 296 | 111 | 23 | 40 | 4.8 |
| | April–May 1966 | 88 | 45 | 11 | 22 | 1.4 |
| Tickle Hr. Point, | May–June 1967 | 245 | 193 | 47 | 46 | 7.3 |
| Trinity Bay | April–May 1968 | 172 | 226 | 9 | 13 | 2.8 |
| Horse Chops, | May–June 1967 | 206 | 191 | 38 | 30 | 6.6 |
| Trinity Bay | April–May 1968 | 156 | 243 | 8 | 3 | 10.0 |
| Cabot Island, | May–June 1967 | 102 | 299 | 29 | 5 | 10.6 |
| Bonavista Bay | May 1968 | 218 | 217 | 0 | 13 | 5.1 |
| Little Denier, | May–June 1967 | 288 | 121 | 51 | 27 | 9.1 |
| Bonavista Bay | May 1968 | 249 | 235 | 1 | 32 | 8.1 |

hydrated condition are present only during the latter half of the spawning interval between successive egg batches.

A comparison of the spawning locations of cod (Fig. 4) with the trajectories of satellite-tracked drifter buoys (Fig. 6) suggests that cod spawn in areas in which eggs and larvae are likely to be retained on the shelf (Helbig et al. (1992) provide further information regarding these trajectories). In general, cod probably avoid spawning on the slope, particularly off Grand Bank, because of the high probability that eggs will be transported away from the banks. Spawning would also be avoided on the southern slopes of Grand Bank and St. Pierre Bank because of potential entrainment, and subsequent mortality, of eggs and larvae in warm core rings (Myers and Drinkwater 1989). Alternatively, spawning should not be unexpected on the Labrador and northeastern Newfoundland slopes because of the existence of eddies that can displace water from the slope onto the shelf (Helbig et al. 1992). The meandering trajectories of buoys on the northeastern Newfoundland shelf and on Grand Bank indicate that eggs spawned in these regions will be retained on the shelf (Fig. 6). Our data, in conjunction with the drifter trajectory routes, are consistent with the hypothesis that spawning locations of marine pelagic fish are dependent upon the geographic extent of oceanographically predictable larval retention areas (O'Boyle et al. 1984).

### Variability in Spawning Distributions: Evidence of Offshore and Inshore Stocks

Disjoint spatial distributions of spawning individuals may lead to or reflect reproductive isolation among cod populations. The spatial distribution of reproductive cod described here might indicate the existence of four such populations on a large scale: a Labrador – northeastern Newfoundland stock, a Grand Bank stock, a Flemish Cap stock, and a St. Pierre stock (cf. Fig. 4). These geographical divisions are consistent with those suggested by Templeman (1962), based upon variation in vertebral numbers (but see Pepin and Carr 1993) and migration studies, and to a lesser extent with the 2J3KL and 3NO stocks identified by Helbig et al. (1992), based upon physical oceanography. Serebryakov et al. (1987) reported a distribution of spawning individuals on Flemish Cap similar to that reported here.

Our data provide evidence of local, inshore populations of cod. Although such evidence is particularly strong in southeastern Newfoundland, an area encompassing that of Templeman's (1962) Avalon–Burin stock, we cannot discount the possibility that spawning also occurs throughout bays in northeastern Newfoundland, areas where ice has prevented comprehensive sampling during spawning. Our conclusion that these spawning inshore cod have not migrated from offshore areas is supported by hydroacoustic survey data reported by Rose (1993). In 1991 and 1992, cod have been monitored during their migration from the north slope of Grand Bank to inshore waters off Bonavista Bay. Sampling indicated that spawning occurred throughout the migration period and that most fish were spent by the time they approached the coast. Thus, the presence of spawning fish in coastal bays is probably indicative of inshore populations, as has been previously postulated by Templeman (1966). Evidence of inshore cod populations also exists in Nova Scotia (McKenzie 1940) and Norway (Bergstad et al. 1987). It is critical for the management of this species that the existence of such populations be explored throughout coastal Newfoundland. Clearly, the gillnet experiments of the 1960s conducted in St. Mary's, Placentia, Trinity, and Bonavista bays should be repeated. Additional surveys should be conducted in

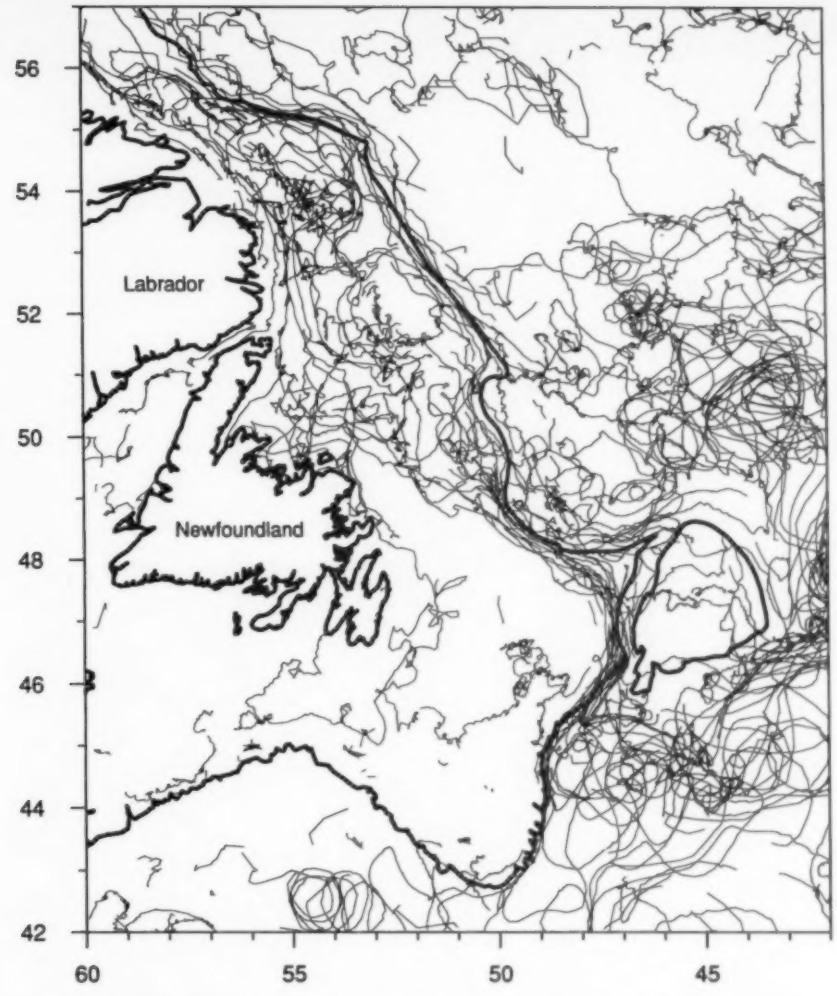



FIG. 6. Trajectories of satellite-tracked drifter buoys off Newfoundland and Labrador. The bold line represents the 1000-m depth contour. Most of the buoys were released on the shelf and slope off Labrador and northeastern Newfoundland; thus the general direction of water flow is from northwest to southeast along the continental slope. Redrawn from Helbig et al. (1992).

Conception, Notre Dame, and White bays. Given the efficient capture of spawning cod inshore by research gill nets (cf. Table 2), questions are raised regarding the degree to which the dramatic rise in the commercial use of gill nets in the early 1960s (Templeman1966, fig. 19) was responsible for the decline in cod stocks in inshore waters since that time. This is particularly relevant given that, in a mixed-population (or species) fishery, those populations with comparatively high catchability become progressively depleted through time (Clark 1985). The existence of inshore populations and their potential importance to recruitment to the fishery warrant study.

## A Historical Review of Cod Spawning Locations in the Newfoundland – Labrador Region

A historical review of the data on cod spawning locations suggests that our data are consistent with information compiled over the past century and that the perception that cod spawn primarily on the slope, perpetuated since the early 1960s, can be attributed primarily to biased sampling.

The first reported spawning locations of cod off Newfoundland and Labrador were inshore areas. Harvey (1891, p. 6) noted that cod could be produced at the newly constructed hatchery at Dildo, Trinity Bay, by crossing "the smaller fish of Trinity Bay" with those near Cape St. Mary "where the largest and finest spawning fish are found early in the season." In fact, broodstock for the hatchery (1890–97) were regularly obtained from the head of Trinity Bay where mature spawning fish "were found in abundance all round Dildo Island" (Nielsen 1895, p. 28) and were collected occasionally from nearby Bull Arm (Nielsen 1894). With regard to the timing of reproduction, Nielsen (1889, cited in Graham 1922) reported that "only during May and June can spawning cod be obtained...in Placentia, Trinity or Conception Bays." Graham (1922) provided limited evidence of spawning cod immediately off St. John's in July and noted the

dearth of research on the spawning of cod inshore prior to 1922.

The first account of possible offshore spawning locations was derived from egg and larval surveys conducted during the Canadian Fisheries Expedition (Biological Board of Canada) in 1914–15 (Dannevig 1919). The area sampled covered much of St. Pierre Bank and the nearby slope. Dannevig (1919) reported the relative abundance of *Gadus* spp. eggs and cod larvae collected from surface hauls (10–15 min in duration) of a 1-m-diameter silk net. Eggs were found on St. Pierre Bank in late May – early June and in late July; larvae were collected primarily during the latter sampling period. Eggs were found over the slope during the former sampling period only.

Frost (1938) summarized the next attempt to identify spawning locations of cod off Newfoundland and Labrador. From 1931 to 1935, gadoid eggs and cod larvae were sampled with surface hauls of 2-m-diameter cheese cloth nets from southeastern Labrador, coastal Newfoundland, and from Grand Bank. Eggs and larvae were collected in abundance within 50 km of the coast in virtually all major bays and were evident throughout much of Grand Bank. Frost's (1938, p.14) conclusion that "some spawning probably occurs on all banks" and Thompson's (1943, p. 90) later interpretation of the same data that "a fair proportion of the total spawning takes place at no great distance from the coast" need to be tempered by the facts that eggs were not identified to species and that larvae may have drifted inshore from spawning locations further offshore.

The third research programme, and the one most influential on later accounts of spawning locations (e.g. Fitzpatrick and Miller 1979; Templeman 1981), was conducted by Soviet researchers. They collected juvenile and adult cod (1957–63) and eggs and larvae (1959–63) along transects which sampled the Newfoundland–Labrador slope far more effectively than the shelf (Serebryakov 1965, 1967). For example, from March to May 1962, the ratio of slope-to-shelf sampling stations off Newfoundland and Labrador increased from 1.3:1 in March and April to 7:1 in May (Serebryakov 1965). This period of ichthyofauna sampling (March–May) would exaggerate the importance of slope versus shelf spawning, and positively bias the importance of the Labrador slope as a spawning location for cod in Newfoundland waters. Myers et al. (1993) have recently documented geographical variation in the timing of cod spawning. A comparison of their estimates of the months in which spawning occurs with those in which the Soviet sampling was conducted reveals that Serebryakov (1965, 1967) would have sampled the Labrador and northeastern Newfoundland slopes during the peak spawning months for this area (March–May) but would have inadequately sampled Grand Bank shelf during 2 of 3 mo of peak spawning (April–June)

Serebryakov (1965, 1967) concluded that spawning took place primarily on the slope off Labrador and northeastern Newfoundland and secondarily on the slopes of Grand Bank. Based upon the locations of egg samples, estimates of current velocity, water temperature, and egg developmental stage duration, he constructed the first map to delineate the areas in which cod were thought to spawn. This figure, flawed by spatial and temporal sampling biasses, was subsequently reproduced by Fitzpatrick and Miller (1979) and has provided the empirical basis for research atlases that are consulted for fisheries management (FAO 1981), the development of offshore oil production (e.g., McGuire 1978), and for models of cod recruitment (Myers and Drinkwater 1988, 1989; Helbig et al. 1992).

Prior to 1963, there had been no concerted attempt to determine the spawning distribution of Newfoundland and Labrador cod by Canadian authorities (e.g., May 1959). It may be noteworthy that the first (and only) cruises with an objective of determining cod spawning times and locations in this region encompassed the 1962 dates of 14–18 April in which Serebryakov (1965, p. 425) reported "large quantities of eggs... on the boundary between central and north Labrador waters on the slope of the shelf over depths 380 to 450 m." The Canadian cruises (*A.T. Cameron* Cruise Reports 68 and 69) of 6–9 April and 30 April – 14 May 1963 were conducted in the same region sampled by the Soviets and also located "large concentrations of spawning and post-spawning cod on the extreme eastern slope of [Hamilton] bank" (Templeman and May 1965, p. 149), although a cruise the following year during the same time period, and sampling the 1963 stations, yielded few spawning or post-spawning individuals (Templeman 1965; *A.T. Cameron* Cruise Report 84). Tagging cruises conducted off Grand Bank in 1964 and 1965 yielded spawning cod on the northeastern, southeastern, and southwestern slopes (*A.T. Cameron* Cruise Reports 85, 102, and 103, respectively); these sampling areas corresponded to those described as "intensive" spawning areas by Serebryakov (1967). Finally, with respect to the perceived importance of the Hamilton Bank slope as the primary cod spawning location, it should be noted that Serebryakov's (1965) totals (pooled for two stations at each location) of 1020 and 650 eggs from the extreme northeastern Labrador slope and the Hamilton Bank slope, respectively, are considerably less than the maximum number of eggs ($n = 1334$) caught in a single haul on St. Pierre Bank in 1914 (Dannevig 1919).

In summary, spawning cod can be found throughout the continental shelf and slope although, with the exception of southeastern Labrador, a small section of northern Grand Bank, and Flemish Cap, spawning occurs primarily on the shelf. The preponderance of shelf spawning is consistent with the hypothesis that cod spawn in areas in which their eggs and larvae are likely to be retained. Our review suggests that there have been historical shifts in the perceived inportance of various spawning locations and that the predominant view of the past 30 yr — offshore spawning being more important than inshore spawning; slope spawning being more prevalent than shelf spawning — lacks empirical support. The absence of representative sampling from the shelf during the years in which present opinions of spawning locations were being formed, and the apparent identification of the slope as the main location of spawning cod in 2J3KL (Templeman 1965; Serebryakov 1965, 1967) appear to be the primary reasons underlying the general belief that cod spawn predominantly on the slope and do so largely east of the Hamilton Bank (e.g., Fitzpatrick and Miller 1979; Templeman 1981; Helbig et al. 1992). Based upon our analysis of all available data, we conclude that offshore spawning occurs primarily on the shelf, particularly off northeastern Newfoundland and on Grand Bank, and that, given the historical consistency of evidence of inshore spawning, coastal populations of cod exist and may have provided a considerably larger contribution to recruitment than has previously been believed.

## Acknowledgements

The research was funded by the Northern Cod Science Programme and could not have been conducted without the extensive sampling undertaken by the Gadoid Section of the Groundfish Division, Department of Fisheries and Oceans, and its predecessors. We thank Joyce Yates for information on historical sources, Jim Helbig, Gordon

Mertz, Joanne Morgan, Ken Frank, and Pierre Pepin for discussion, and Nicholas Barrowman for assistance in preparing the figures. Robin Anderson, Nicholas Barrowman, Chris Chambers, Gordon Mertz, George Rose, Joanne Morgan, Ed Trippel, and an anonymous reviewer provided helpful comments on an earlier version of the manuscript.

## References

BAIRD, J.W., C.A. BISHOP, W.B. BRODIE, AND E.F. MURPHY. 1992. An assessment of the cod stock in NAFO divisions 2J3KL. NAFO SCR Doc. 92/18, Ser. No. N2063: 69 p.

BERGSTAD, O.A., T. JØRGENSEN, AND O. DRAGESUND. 1987. Life history and ecology of the gadoid resources of the Barents Sea. Fish. Res. 5: 119–161.

BRAWN, V.M. 1961. Reproductive behaviour of the cod (*Gadus callarias* L.). Behaviour 18: 177–197.

CLARK, C.W. 1985. Bioeconomic modelling and fisheries management. John Wiley & Sons, New York, N.Y.

DANNEVIG, A. 1919. Canadian fish-eggs and larvae, p. 1–79. *In* Canadian Fisheries Expedition, 1914–1915, Investigations in the Gulf of St. Lawrence and Atlantic waters of Canada. King's Printer, Ottawa, Ont.

DE YOUNG, B., AND G.A. ROSE. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can J. Fish. Aquat. Sci. 50. (In press)

FAO. 1981. Atlas of the living resources of the seas. Food and Agriculture Organization, United Nations, Rome, Italy.

FITZPATRICK, C., AND R.J. MILLER. 1979. Review of spawning times and locations for some commercial finfish on the Newfoundland and Labrador coasts. Fish. Mar. Serv. Tech. Rep. 905: 14 p.

FROST, N. 1938. Some fishes of Newfoundland waters. Res. Bull. No. 4, Department of Natural Resources (Newfoundland Government), St. John's, Nfld. 16 p.

GAVARIS, S., AND W.B. BRODIE. 1984. Results of comparative fishing between the *A.T. Cameron* and the *Wilfred Templeman* during July–August 1983. CAFSAC Res. Doc. 84/41: 16 p.

GRAHAM, M. 1922. Investigations comprising observations on the natural history of the Newfoundland shore cod. Report to the Director of Fishery Investigations, Fisheries Laboratory, Lowestoft, U.K.

HARVEY, A.W. 1891. Operations at Dildo, p. 5–12. *In* Annual report of the Newfoundland Fisheries Commission for the year 1890. J.W. Withers, St. John's, Nfld.

HELBIG, J., G. MERTZ, AND P. PEPIN. 1992. Environmental influences on the recruitment of Newfoundland/Labrador cod. Fish. Oceanogr. 1: 39–56.

KJESBU, O.S., P.R. WHITTHAMES, P. SOLEMDAL, AND M. GREER WALKER. 1990. Ovulatory rhythm and a method to determine the stage of spawning in Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 47: 1185–1193.

MANLY, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, New York, N.Y.

MAY, A.W. 1959. Cod investigations in subarea 2 — Labrador, 1950 to 1958. ICNAF Annu. Proc. 9: 103–105.

MCGUIRE, B.E. 1978. Environmental atlas of coastal Labrador. Environmental Emergency Division, Environmental Protection Service, Atlantic Region, Halifax, N.S.

MCKENZIE, R.A. 1940. Nova Scotian autumn cod spawning. J. Fish. Res. Board Can. 5: 105–120.

MYERS, R.A., AND K.F. DRINKWATER. 1988. Offshelf Ekman transport and larval fish survival in the northwest Atlantic. Biol. Oceanogr. 6: 45–64.

MYERS, R.A., AND K.F. DRINKWATER. 1989. The influence of Gulf Stream warm core rings on recruitment of fish in the northwest Atlantic. J. Mar. Res. 47: 635–656.

MYERS, R.A., G. MERTZ, AND C.A. BISHOP. 1993. Cod spawning in relation to physical and biological cycles of the northern Northwest Atlantic. Fish. Oceanogr. 2: 154–165.

NIELSEN, A. 1889. *In* Annual report of the Newfoundland Department of Fisheries, for the year 1888. St. John's, Newfoundland.

NIELSEN, A. 1894. Operations at Dildo Island Hatchery, p. 20–35. *In* Annual report of the Newfoundland Department of Fisheries, for the year 1893. J.W. Withers, St. John's. Nfld.

NIELSEN, A. 1895. Operations at Dildo Island Marine Hatchery, p. 26–48. *In* Annual report of the Newfoundland Department of Fisheries, for the year 1894. J.W. Withers, St. John's, Nfld.

O'BOYLE, R.N., M. SINCLAIR, R.J. CONOVER, K.H. MANN, AND A.C. KOHLER. 1984. Temporal and spatial distribution of ichthyoplankton communities of the Scotian Shelf in relation to biological, hydrological, and physiographic features. Rapp. P.-v. Réun. Cons. int. Explor. Mer 183: 27–40.

PEPIN, P., AND S.M. CARR. 1993. Morphological, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland shelf. Can. J. Fish. Aquat. Sci. 50: 1924–1933.

ROSE, G.A. 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature (Lond.) 366: 458–461.

SEREBRYAKOV, V.P. 1965. Some results of Soviet research work on ichthyoplankton in the Northwest Atlantic: eggs and larvae of cod. ICNAF Spec. Publ. No. 6: 425–433.

SEREBRYAKOV, V.P. 1967. Cod reproduction in Northwest Atlantic. Tr. PINRO 20: 205–242. (Transl. from Russian by Fish. Res. Board Can. 1133, 1968)

SEREBRYAKOV, V.P., A.V. ASTAFJEVA, V.K. ALDONOV, AND A.K. CHUMAKOV. 1987. USSR ichthyoplankton investigations on Flemish Cap, 1978–83. NAFO Sci. Coun. Stud. 11: 7–21.

TEMPLEMAN, W. 1962. Divisions of cod stocks in the Northwest Atlantic. ICNAF Redbook 1962, Part III: 79–129.

TEMPLEMAN, W. 1965. Canadian research report 1964. A. Subareas 2 and 3. ICNAF Redbook 1965, Part II: 3–20.

TEMPLEMAN, W. 1966. Marine Resources of Newfoundland. Bull. Fish. Res. Board Can. 154: 170 p.

TEMPLEMAN, W. 1981. Vertebral numbers in Atlantic cod, *Gadus morhua*, of the Newfoundland and adjacent areas, 1947–1971, and their use for delineating cod stocks. J. Northwest Atl. Fish. Sci. 2: 21–45.

TEMPLEMAN, W., V.M. HODDER, AND R. WELLS. 1978. Sexual maturity and spawning in haddock, *Melanogrammus aeglefinus*, of the southern Grand Bank. ICNAF Res. Bull. No. 13: 53–65.

TEMPLEMAN, W., AND A.W. MAY. 1965. Research vessel catches of cod in the Hamilton Inlet Bank area in relation to depth and temperature. ICNAF Spec. Publ. No. 6: 149–165.

THOMPSON, H. 1943. A biological and economic study of cod (*Gadus callarias*, L.). Res. Bull. No. 14, Department of Natural Resources (Newfoundland Government), St. John's, Nfld. 160 p.

# Effect of Age on the Seasonality of Maturation and Spawning of Atlantic Cod, *Gadus morhua*, in the Northwest Atlantic

Jeffrey A. Hutchings and Ransom A. Myers

*Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's NF A1C 5X1, Canada*

Hutchings, J.A., and R.A. Myers. 1993. Effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 50: 2468–2474.

Analysis of 46 yr of research trawl survey data from seven regions in the Northwest Atlantic (Newfoundland and Labrador) indicated that older individuals of Atlantic cod, *Gadus morhua*, of both sexes initiated and completed spawning later, and spawned for a greater length of time, than younger individuals. Within regions, males were in spawning condition for longer periods than females. Among regions, spawning duration of males (age = 11 yr) averaged 43.5 ± 13.6 d and was significantly longer than that of females (age = 11 yr; 25.2 ± 8.4 d). The strong association between age and time of spawning provides empirical evidence of size-specific, assortative mating in cod. The decline in recruitment of cod in NAFO Division 2J3KL since 1962 is associated with a dramatic reduction in the fecundity contribution of older (15–20 yr) relative to younger (7–9 yr) individuals. Our results indicate that this increase in proportional abundance of younger individuals should be concomitant with a decline in the duration of spawning time, reducing the probability that larval emergence will match peak abundances of zooplankton. Thus, age dependence of maturation and spawning times provides a mechanism by which size-selective mortality against larger, older individuals can increase variability in recruitment in Atlantic cod.

L'analyse des données de campagne de chalutage portant sur une période de 46 ans et provenant de sept régions dans le nord-ouest de l'Atlantique (Terre-Neuve et Labrador) a montré que des individus âgés de morue franche, *Gadus morhua*, des deux sexes entreprenaient et terminaient leur période de fraye plus tard, et frayaient plus longtemps que de jeunes individus. Dans les régions, les mâles étaient prêts à frayer pendant des périodes plus longues que les femelles. D'une région à l'autre, la durée de la fraye chez les mâles (âge = 11 ans) s'établissait en moyenne à 43,5 ± 13,6 jours et elle était significativement plus longue que chez les femelles (âge = 11 ans; 25,2 ± 8,4 jours). La forte association entre l'âge et la durée de la fraye prouve de façon empirique qu'il y a accouplement par sélection selon la taille chez la morue. La baisse du recrutement chez la morue de la division 2J3KL de l'OPANO depuis 1962 est associée à une baisse spectaculaire de la fécondité des vieux individus (15 à 20 ans) par rapport aux jeunes individus (7 à 9 ans). Nos résultats montrent que cette augmentation de l'abondance proportionnelle de jeunes individus devrait être concomitante à une baisse de la durée de la fraye, ce qui réduit la probabilité que l'émergence des larves corresponde aux abondances maximales du zooplancton. La période de maturation et de fraye selon l'âges fournit donc un mécanisme selon lequel la mortalité selon la taille par rapport à des individus plus âgés et plus gros peut augmenter la variabilité du recrutement chez la morue franche.

*Received February 3, 1993*
*Accepted May 31, 1993*
*(JB778)*

*Reçu le 3 février 1993*
*Accepté le 31 mai 1993*

Temporal variation in the timing of reproduction represents a potentially important source of variability in the recruitment of juveniles to the adult portion of any fish population. The physical environment, for example, might delay or unduly extend the spawning period through temperature-induced constraints on the migration of adults to spawning habitat or on the physiological processes associated with maturation. Individual factors such as age and body size can also influence the timing of reproduction. If it is advantageous to spawn as early as possible in the spawning season, older females may spawn earlier than younger females because of sexual selection, by males, for large body size (e.g., kokanee salmon, *Oncorhynchus nerka*. Foote 1988). Similarly, smaller female salmonids may delay reproduction until larger females have spawned to reduce egg mortality by egg nest superimposition (van den Berghe and Gross 1984; Myers 1986). Alternatively, larger individuals may reproduce later than smaller individuals because of longer maturation periods effected by generally slower metabolic rates or by greater, size-independent energy costs (Peters 1983).

Age-specific differences in the rate of gonadal development or in the duration of spawning should increase recruitment variability when the age-specific survival of adults varies from one generation to the next. Variability in survival is common in commercially exploited populations. Recruitment of Atlantic cod, *Gadus morhua* (as determined by virtual population analysis; Baird et al. 1992), in Northwest Atlantic Fisheries Organization (NAFO) Division 2J3KL off Labrador and northern Newfoundland varied by almost an order of magnitude from 1962 to 1988 (Fig. 1A). The temporal decline in cod abundance was accompanied by an overall 85% reduction in the total fecundity of old individuals (15–20 yr) relative to the total fecundity of young individuals (7–9 yr) (Fig. 1B) (age ranges were chosen simply to be representative of old and young mature fish). This reduction may be indicative of a progressive decrease in the proportion of older females in the spawning population and a concomitant narrowing of the variation in age among reproductive individuals. If age significantly influences the timing of reproduction, then a decline in the number of age classes during spawning

may shorten the duration of the spawning season and reduce the likelihood that larvae begin feeding during the period of peak zooplankton abundance. Our objective is to use research trawl data, collected over 46 yr, to determine the influence of age on the timing of maturation and spawning in male and female Atlantic cod.

## Materials and Methods

Spawning times were calculated from research trawl surveys conducted between 1946 and 1991 by the Canadian Department of Fisheries and Oceans and its predecessors. Prior to the early 1970's, cod were sampled systematically by line transect surveys. Surveys undertaken since that time were randomly stratified by depth. We combined all research surveys for our analyses. Data were analyzed for each of seven geographical areas in the Newfoundland/Labrador Region (Fig. 2). These areas included NAFO Divisions 3L, 3N, 3O, and 3Ps, in entirety. Divisions 4R and 4S in the northern Gulf of St. Lawrence were combined (4RS). Data from Divisions 2J and 3K were represented by Belle Isle Bank in the north and by Funk Island Bank in the south. Flemish Cap (NAFO Division 3M), Hamilton Bank (2J), and the northern coast of Labrador (2GH) were excluded from the analyses because of insufficient data. Ice cover restricted extensive sampling during spawning in Belle Isle and Funk Island banks. Myers et al. (1993) provided a summary of the monthly samples available from these regions.

Individuals were analyzed by sex and by maturation stage (following Templeman et al. 1978) (Table 1). Females were divided into three stages: *maturing* (eggs opaque and visible to naked eye), *spawning* (ovary containing clear eggs, clarity being indicative of hydration and readiness for release; cf. Kjesbu 1989; Rijnsdorp 1989), and *spent* (ovaries whitish-grey or bluish-grey, slack, and often wrinkled; residual eggs often present). The three maturation stages for males were *maturing* (colour of testes ranging from gray or pink to white; little or no sperm in the vas deferentia), *spawning* (testes and vas deferentia white and full of milt), and *spent* (testes grey or pink; milt not evident in outer edges at the time that all females are spent).

To examine the influence of age on the timing of a given maturation stage, all individuals whose gonads were in a developmental state preceding the stage of interest were classified 0 and all remaining individuals were classified 1. Age determinations were calculated from otoliths. We used a maximum likelihood probit analysis to analyze the effects of age on the timing of two maturity states (spawning, spent). The probability, $p$, of an individual maturing at age $a$ years on day of year $t$ was assumed to be

$$(1) \quad p = \Phi(\beta_0 + \beta_1 t + \beta_2 a)$$

where $\Phi$ is the cumulative distribution function for the standard normal distribution and $\beta_0$, $\beta_1$, and $\beta_2$ are the probit regression parameters. For a given $t$, the standard deviation of the timing of each stage at a given age $a$ is $1/\beta_2$. Following the PROC PROBIT procedure in SAS (SAS Institute 1989), the standard errors of the parameters were inflated by a heterogeneity factor if there was a lack of fit caused by extrabinomial variation. This extrabinomial variation is expected because of cluster sampling from trawls and perhaps microgeographic or interannual variability in maturity.

We defined the times of initiation and cessation of spawning

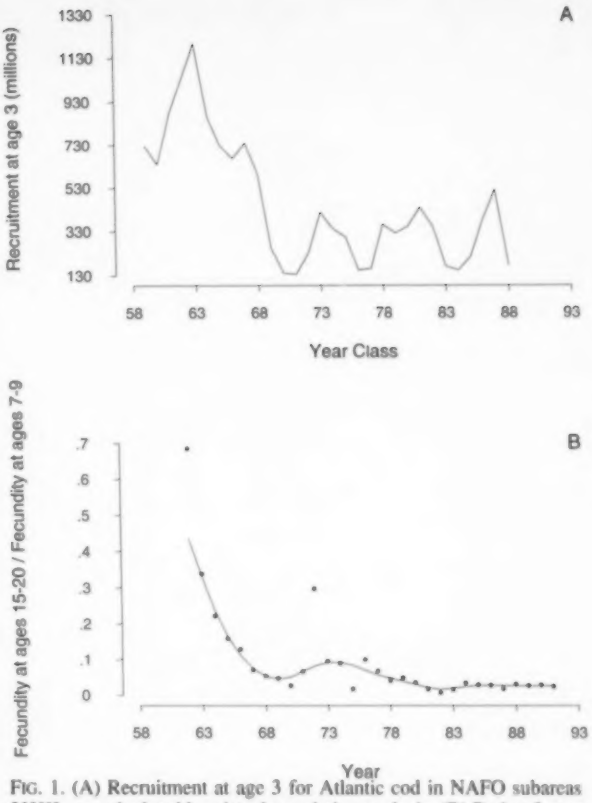



FIG. 1. (A) Recruitment at age 3 for Atlantic cod in NAFO subareas 2J3KL, as calculated by virtual population analysis. (B) Ratio of eggs produced by older cod (ages 15–20) to those produced by younger cod (ages 7–9). The fecundity at age data were calculated from May (1967) and the numbers at age were estimated from catch at age data (Baird et al. 1992). The observations were smoothed by an algorithm that uses running medians (cf. Tukey 1977).

of individuals aged $a$ years as the day of year, $t$, on which 50% of the fish were expected to be in a spawning or spent condition, respectively. Thus, given that $0.5 = \Phi(0)$, times of spawning initiation and cessation can be calculated as

$$(2) \quad t = (-\beta_0 - \beta_2 a) / \beta_1.$$

Spawning duration was defined as the difference in times of spawning initiation and spawning cessation.

## Results

Age had a significantly positive effect on the initiation and cessation of spawning for males and females in all regions (Table 2). Older individuals of both sexes completed spawning later than younger individuals (Fig. 3 and 4). Younger individuals began spawning before older individuals in all regions although the age effect was not significant for either males or females in 3L and 4RS.

The slopes of the probit regressions relating age to the cessation of spawning were greater than the slopes of the regressions between age and the initiation of spawning in all but two cases (Fig 3 and 4). The probability of obtaining 12 such deviations

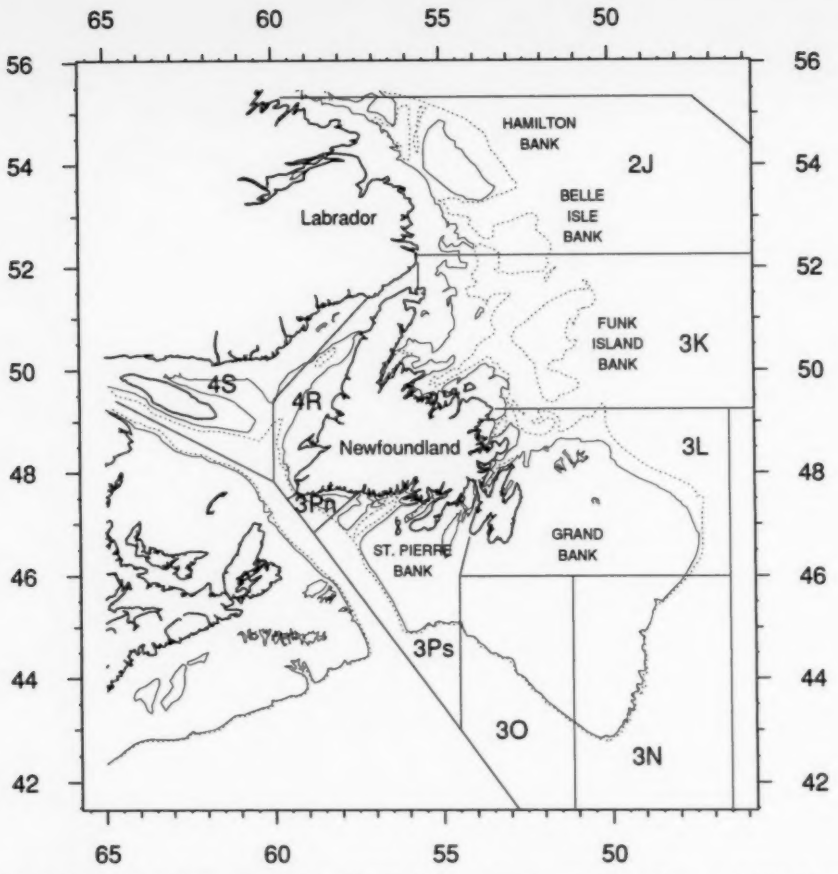



FIG. 2. Map of the study region, including NAFO divisions. Depth contours are 200 m (solid lines) and 300 m (broken lines).

TABLE 1. Sample sizes of Atlantic cod in various reproductive states (see text for explanation) in research trawls conducted off Newfoundland from 1946 to 1991.

| | Females | | | Males | | |
|---|---|---|---|---|---|---|
| Region | Maturing | Spawning | Spent | Maturing | Spawning | Spent |
| Belle Isle | 1360 | 118 | 14263 | 1279 | 335 | 14133 |
| Funk Island | 1328 | 99 | 3973 | 1385 | 191 | 2475 |
| 3L | 2928 | 1167 | 5480 | 3438 | 1832 | 6289 |
| 3N | 1073 | 209 | 1574 | 1149 | 416 | 4760 |
| 3O | 1447 | 299 | 1070 | 1492 | 557 | 1237 |
| 3Ps | 2376 | 249 | 764 | 2926 | 396 | 817 |
| 4RS | 1164 | 119 | 2099 | 4855 | 338 | 6877 |

among the 14 cases observed is significantly greater than expected by chance alone (sign test; $t_s = 3.77 > t_{0.01[13]} = 3.01$). This slope divergence, which was more evident among males, indicates that older individuals have a longer reproductive period than younger individuals. The two regions in which the slopes did not diverge for females (Belle Isle and Funk Island banks) were characterized by disproportionately low sample sizes of spawning individuals (Table 1).

Spawning duration for males aged 11 (the midrange of 6–16 yr) (range: 22 d in 3N to 57 d in 4RS) was longer than that for similarly aged females (range: 6 d in Belle Isle Bank to 32 d in 3O) within each region. Among regions, the spawning duration of males aged 11 yr (38.6 ± 13.8 d) was significantly greater than that for females (19.8 ± 11.5 d: $F_{[1,12]} = 7.58$; $p = 0.017$, $p$ was assessed from 1000 data randomizations; cf. Manly 1991). If the two regions for which sample sizes of spawning individuals were disproportionately small (Belle Isle and Funk Island banks) are excluded, spawning duration of 11-yr-old

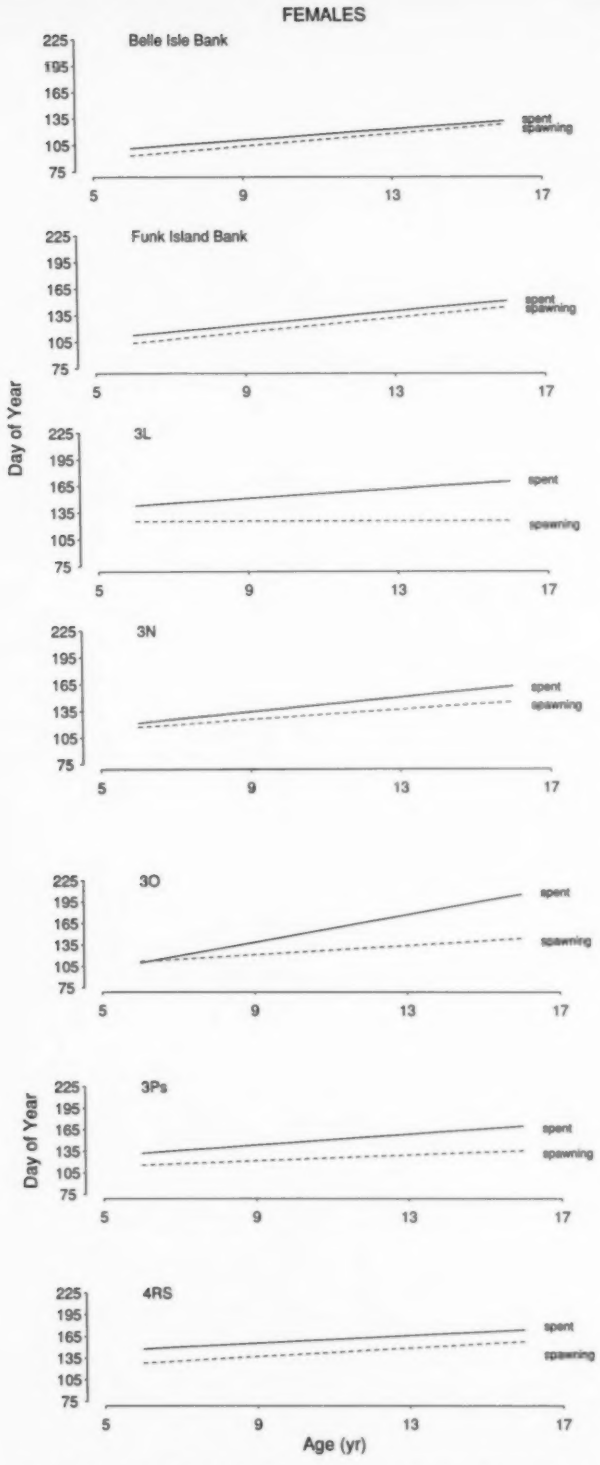



FIG. 3. Estimated relationship between age and the day of year at which 50% of female Atlantic cod enter into spawning and spent maturation states. See text for details.

TABLE 2. Probit regression coefficients ($\beta_2$ in Eq. 1) of the effects of age on the timing of reproduction in female and male Atlantic cod (standard errors in parentheses). *$0.01 < p < 0.05$; **$0.001 < p < 0.01$; *** $p < 0.001$.

| | Females | | Males | |
|---|---|---|---|---|
| Region | Spawning | Spent | Spawning | Spent |
| Belle Isle | –0.135*** | –0.129*** | –0.144*** | –0.201*** |
| | (0.013) | (0.013) | (0.015) | (0.040) |
| Funk Island | –0.120*** | –0.133*** | –0.133*** | –0.203*** |
| | (0.019) | (0.017) | (0.017) | (0.022) |
| 3L | 0.001 | –0.064*** | –0.008 | –0.070*** |
| | (0.007) | (0.008) | (0.010) | (0.012) |
| 3N | –0.108*** | –0.155*** | –0.054*** | –0.149*** |
| | (0.009) | (0.017) | (0.009) | (0.011) |
| 3O | –0.101*** | –0.223*** | –0.065*** | –0.200*** |
| | (0.011) | (0.028) | (0.009) | (0.017) |
| 3Ps | –0.050*** | –0.077*** | –0.060*** | –0.108*** |
| | (0.012) | (0.014) | (0.013) | (0.023) |
| 4RS | –0.033 | –0.053* | –0.054 | –0.075** |
| | (0.020) | (0.021) | (0.039) | (0.027) |

males and females increases to 43.5 ± 13.6 and 25.2 ± 8.4 d, respectively, and remains significantly different between sexes ($F_{[1,8]} = 6.52$; $p = 0.034$).

## Discussion

The classical explanation of the timing of reproduction in marine pelagic fish is given by Cushing's (1969) match–mismatch hypothesis. This model assumes that spawning time is constrained by the necessity of maximizing the overlap between larval abundance and the seasonal supply of zooplankton. If this hypothesis is true, our results suggest that the timing of the peak of larval abundance is not precise because of age-specific effects on the timing of reproduction. We found that age has a positive effect on the timing of maturation and spawning in male and female Atlantic cod. Although at variance with long-accepted notions in the literature (e.g., Jónsson 1961; Harden Jones 1968), our results are consistent with studies for which empirical data on maturation are available (e.g., Jónsson 1982).

Ellertsen et al. (1989, p. 216), citing Sorokin's (1957) discussion of the Barents Sea cod stock, stated that "it is well known that larger and older fish spawn earlier than first time spawners." With respect to the Icelandic cod stocks, Jónsson (1961, p. 131) also remarked that "it is a well known fact, that the first-time spawners start spawning later in the season than the older ones" although he provided no data to support this assertion. The conclusion that older cod spawn earlier than younger cod is based primarily on commercial fish catch data from Lofoten, Norway, the main spawning area for the Barents Sea population (Pedersen 1984). These data (Sund 1938; Rollefsen 1940) indicated that the size of cod in commercial catches declined from mid-February through March. While these data suggest that older fish arrive on the spawning grounds before younger fish, it seems unwarranted to then conclude that older cod spawn before younger cod. According to Bergstad et al. (1987), spawning in Lofoten occurs primarily from mid-March through mid-April. Given that fish of all sizes and ages are present in the Kabelvåg, Sørvågen, Balstad, and Værøy regions of Lofoten by mid-to late March (Sund 1938; Rollefsen 1940), the data do not imply that time of spawning is

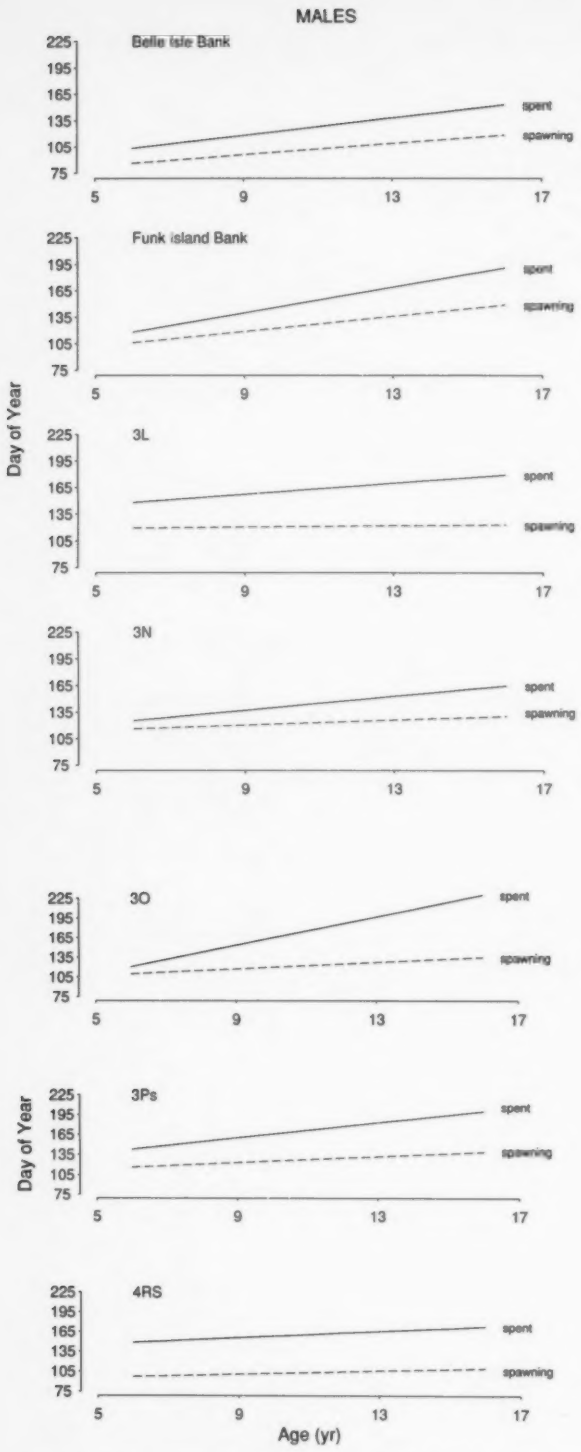



FIG. 4. Estimated relationship between age and the mean day of year at which 50% of male Atlantic cod enter into spawning and spent maturation states. See text for details.

inversely related to age.

Our conclusion that age is positively associated with spawning time is supported by Jónsson's (1982) maturity data on Icelandic cod. He combined data from 1953 to 1974 and documented a progressive increase in the proportional representation of older spawning fish (sexes combined) throughout the spawning period. The relative importance of his suggestion that young spawners also spawn near the end of the spawning period is difficult to assess because he did not report age- and size-specific sample sizes. Recent spermatological data on spawning cod from NAFO region 3L collected by E.A. Trippel and M.J. Morgan (Department of Fisheries and Oceans, Biological Station, St. Andrews, NB E0G 2X0, Canada, and Department of Fisheries and Oceans, P.O. Box 5661, St. John's, NF A1C 5X1, Canada, personal communications) indicated that younger males had depleted or spent their sperm volume before older males. These data provide empirical evidence of the positive association between age and time of spawning that we documented for Newfoundland/Labrador region cod. The negative correlation between age and spawning time that has been documented in herring, *Clupea harengus* (Iles 1964; Lambert 1987), and in brown trout, *Salmo trutta* (Elliott 1984), may reflect size-dependent, competitive interactions associated with spawning on a substrate. Although laboratory work indicates that some hydrated eggs are always present in females once they have begun to spawn (Kjesbu 1988), it is possible that some of the females we classified as maturing females (because of an absence of hyaline eggs) may have been spawning. However, the similarity of the age-related effects on spawning between females and males (whose maturation states are more easily identifiable) suggests that any misclassifications were unlikely to bias our results.

The data suggest that the duration of spawning increases with increasing age for both male and female cod. Such an association may or may not be adaptive. There can be considerable selective advantages to spreading reproduction over time to reduce temporal variance in offspring production (Gillespie 1977). By spawning over a wider seasonal window, an individual reduces the probability of having its offspring experience suboptimal conditions for growth and survival, e.g., entrainment off the continental shelf by Gulf stream warm core rings (Myers and Drinkwater 1988). Alternatively, physical factors may effect lengthened spawning by older individuals. Given that females release their eggs in multiple batches and that the interval between batches is approximately constant (Kjesbu 1989), larger females would be expected to spawn over a longer period of time than smaller females simply because of the former's greater fecundity (May 1967) (unless larger females extrude more eggs per batch). A second, nonadaptive explanation is that space limitations within a female's body prevent the hydration of her entire complement of eggs at one time. Thus, larger females would be forced to hydrate their eggs over a longer period of time than smaller females.

The reliability of our data can be assessed by comparing our indirect estimates of spawning duration with estimates measured in the laboratory. Excluding Belle Isle and Funk Island banks, spawning duration for female cod averaged 25 d. Kjesbu (1989) documented spawning intervals of 47, 51, and 60 d for three female Norwegian cod reproducing in tanks at 5–8°C. However, given that the cod in this study were approximately 2.5 times as fecund as those reared in the wild (Kjesbu 1989), the expected spawning intervals of cod in the wild, calculated by reducing

Kjesbu's (1989) estimates by 2.5, would be 19, 20, and 24 d — estimates similar to ours. K. Waiwood and C. Chambers (Department of Fisheries and Oceans, Biological Station, St. Andrews, NB E0G 2X0, Canada, and Huntsman Marine Science Centre, St. Andrews, NB, E0G 2X0, Canada, personal communications) documented a median spawning duration of 30–34 d for 10 Northwest Atlantic female cod at 4°C in the laboratory. Egg surveys from 1976 to 1982 in Lofoten, Norway (Pedersen 1984; see also Ellertsen et al. 1989), provide additional evidence that 25–35 d is a reasonable estimate of individual spawning duration. The presence of newly hatched eggs (less than 2 d old) in Lofoten is restricted to a relatively short period of time. Using the data provided by Pedersen (1984), we calculated the standard deviation of the duration of newly hatched eggs to be only 10.4 d. As this parameter incorporates variability among all spawning individuals, it is unlikely that the spawning duration of any single individual in Lofoten will, on average, be much greater than 30 d. In summary, the similarity of our estimates of spawning duration with those measured in the laboratory and estimated from egg surveys suggests that our data represent reasonable approximations of reproduction in the wild.

Our finding that both younger males and younger females initiate and complete spawning prior to their older counterparts provides indirect empirical evidence of assortative mating in cod. A strong argument for assortative mating can be made from the behavioural data provided by Brawn (1961) in her description of cod spawning in the laboratory. She noted that, after mounting the female dorsally, the male slips down on one side of the female, clasping her with his pelvic fins, and lies "in an inverted position below the female with the ventral surfaces of both fish and their genital apertures closely pressed together" (Brawn 1961, p. 187). Clearly, the probability of egg fertilization will be maximized when the vents of the spawning pair coincide, as would be the case for individuals of the same size.

The delay in spawning of older individuals, relative to younger individuals, may simply be the consequence of a delay in maturation. If the rate of production of gonadal tissue is largely independent of body size, as would be expected (Calder 1984) at the low temperatures −1 to 3°C) at which Newfoundland/Labrador cod mature, then larger, older fish should take longer to produce their entire volume of ova or sperm than smaller, younger individuals because larger fish produce more eggs and sperm than smaller fish.

Our results provide strong evidence that the duration of spawning increases with age. The relative abundance of older cod in NAFO subareas 2J3KL has decreased dramatically since 1962, suggesting that the duration of spawning has also declined in this population (cf. Fig. 1). Given that increased fishing mortality will generally reduce the proportion of older fish in a population, this may increase variability in recruitment by decreasing the average age of spawning. Such an increase in recruitment variability has been hypothesized to exist at low stock sizes (Daan 1978; Shepherd and Cushing 1990). Increased proportional representation of younger individuals in the spawning population should reduce variability both in spawning times and in the temporal presence of larvae in early life. The latter will reduce the probability that larval emergence will coincide with peak abundances of zooplankton. The age dependence of maturation and spawning demonstrated here provides a mechanism by which size-selective mortality against larger, older individuals can increase variability in recruitment in Atlantic cod.

## Acknowledgements

The research was funded by the Northern Cod Science Programme and could not have been conducted without the extensive sampling undertaken by the Gadoid Section of the Groundfish Division, Department of Fisheries and Oceans, and its predecessors. We are grateful to Ed Trippel, Joanne Morgan, Ken Waiwood, and Chris Chambers for discussion and for their permission to cite unpublished data. We thank Claude Bishop, Ken Drinkwater, Terje Jørgensen, Joanne Morgan, Adriaan Rijnsdorp, Svein Sundby, and an anonymous reviewer for helpful comments on earlier versions of the manuscript.

## References

BAIRD, J.W., C.A. BISHOP, W.B. BRODIE, AND E.F. MURPHY. 1992. An assessment of the cod stock in NAFO divisions 2J3KL. CAFSAC Res. Doc. 92/75: 76 p.

BERGSTAD, O.A., T. JØRGENSEN, AND O. DRAGESUND. 1987. Life history and ecology of the gadoid resources of the Barents Sea. Fish. Res. 5: 119–161.

BRAWN, V.M. 1961. Reproductive behaviour of the cod (*Gadus callarias* L.) Behaviour 18: 177–197.

CALDER, W.A. 1984. Size, function and life history. Harvard University Press, Cambridge, Mass. 431 p.

CUSHING, D.H. 1969. The regularity of the spawning season of some fishes. J. Cons. int. Explor. Mer 33: 81–97.

DAAN, N. 1978. Changes in cod stocks and cod fisheries in the North Sea. Rapp. P.-v. Réun. Cons. int. Explor. Mer 172: 39–57.

ELLERTSEN, B., P. FOSSUM, P. SOLEMDAL, AND S. SUNDBY. 1989. Relation between temperature and survival of eggs and first-feeding larvae of northeast Arctic cod (*Gadus morhua* L.) Rapp. P.-v. Réun. Cons. int. Explor. Mer 191: 209–219.

ELLIOTT, J.M. 1984. Numerical changes and population regulation in young migratory trout *Salmo trutta* in a Lake District stream, 1966–1983. J. Anim. Ecol. 53: 327–350.

FOOTE, C.J. 1988. Male mate choice dependent on male size in salmon. Behaviour 106: 63–80.

GILLESPIE, J.H. 1977. Natural selection for variances in offspring numbers: a new evolutionary principle. Am. Nat 111: 1010–1014.

HARDEN JONES, F.R. 1968. Fish migration. Edward Arnold, London. 325 p.

ILES T.D. 1964. The duration of maturation stages in herring. J. Cons. int. Explor. Mer 29: 166–188.

JÓNSSON, J. 1961. On the spawning stocks of cod in East Greenland and Iceland waters in 1959. Ann. Biol. 16: 130–135.

JÓNSSON, E. 1982. A survey of spawning and reproduction of the Icelandic cod. Rit. Fiskideildar 6: 45 p.

KJESBU, O.S. 1988. Aspects of the reproduction in cod (*Gadus morhua* L.): oogenesis, fecundity, spawning in captivity and stage of spawning. Ph.D. thesis, University of Bergen, Bergen, Norway. 147 p.

KJESBU, O.S. 1989. The spawning activity of cod, *Gadus morhua* L. J. Fish Biol. 34: 195–206.

LAMBERT, T.C. 1987. Duration and intensity of spawning in herring *Clupea harengus* as related to the age structure of the mature population. Mar. Ecol. Prog. Ser. 39: 209–220.

MANLY, B.F.J. 1991. Randomization and Monte Carlo methods in biology. Chapman & Hall, New York, N.Y. 281 p.

MAY, A.W. 1967. Fecundity of Atlantic cod. J. Fish. Res. Board Can. 24: 1531–1551.

MYERS, R.A. 1986. Game theory and the evolution of Atlantic salmon (*Salmo salar*) age at maturation, p. 53–61. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

MYERS, R.A., AND K.F. DRINKWATER. 1988. Offshelf Ekman transport and larval fish survival in the northwest Atlantic. Biol. Oceanogr. 6: 45–64.

MYERS, R.A., G. MERTZ, AND C.A. BISHOP. 1993. Cod spawning in relation to physical and biological cycles of the northern Northwest Atlantic. Fish. Oceanogr. 2: 154–165.

PEDERSEN, T. 1984. Variation of peak spawning of Arcto-Norwegian cod (*Gadus morhua* L.) during the time period 1929–1982 based on indices estimated from fishery statistics, p. 301–316. *In* E. Dahl, D.S. Danielssen, E. Moksness, and P. Solemdal [ed.] The propagation of cod *Gadus morhua* L. Flødevigen Rapportser 1, Arendal, Norway.

PETERS, R.H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge, Mass. 329 p.

RIJNSDORP, A.D. 1989. Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.) J. Cons. int. Explor. Mer 46: 35–51.

ROLLEFSEN, G. 1940. Skreibestanden 1939. FiskDir. Skr. Ser. HavUnders. 76: 69–84. (In Norwegian)

SAS INSTITUTE. 1989. SAS/STAT user's guide, version 6, 4th ed., Vol. 2. SAS Institute Inc., Cary, N.C. 846 p.

SHEPHERD, J.G., AND D.H. CUSHING. 1990. Regulation in fish populations: myth or mirage? Philos. Trans. R. Soc. Lond. B 330: 151–164.

SOROKIN, V.P. 1957. Oogenesis and sexual cycle of cod (*Gadus morhua* L.) Tr. PINRO 10: 125–144.

SUND, O. 1938. Torskebestanden i 1937. FiskDir. Skr. Ser. HavUnders. 5: 11–22. (In Norwegian)

TEMPLEMAN, W., V.M. HODDER, AND R. WELLS. 1978. Sexual maturity and spawning in haddock, *Melanogrammus aeglefinus*, of the southern Grand bank. ICNAF Res. Bull. No. 13: 53–65.

TUKEY, J.W. 1977. Exploratory data analysis. Addison-Welsey, Reading, Mass. 688 p.

VAN DEN BERGHE, E.P., AND M.R. GROSS. 1984. Female size and nest depth in coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 41: 204–206.

# Scale Characteristics of Wild and Hatchery Chinook Salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, New Zealand, and Their Use in Stock Identification

M.J. Unwin and D.H. Lucas

*NIWA Freshwater, National Institute of Water and Atmospheric Research, P.O. Box 8602, Christchurch, New Zealand*

Unwin, M.J., and D.H. Lucas. 1993. Scale characteristics of wild and hatchery chinook salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, New Zealand, and their use in stock identification. Can. J. Fish. Aquat. Sci. 50: 2475–2484.

To distinguish between fish with potentially desirable behavioural or genetic traits, we examined scales from wild and hatchery chinook salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, New Zealand, and a tributary, Glenariffe Stream. Scales from wild chinook with a "stream-type" life history corresponded closely to those from 1+ juveniles, but the mean size of the freshwater zone for scales from "ocean-type" adults was significantly larger than for age 0+ juveniles. Scales from fish of hatchery origin lacked a distinct freshwater zone but were distinguishable from scales from wild fish by the location of the first annulus. Linear discriminant function analysis (DFA) based on the location of the first annulus correctly identified 82–90% of the fish in a mixed adult population, increasing to 88–92% when fork length and age were included. Estimates of the number of hatchery fish entering Glenariffe Stream from 1989 to 1992, based on DFA, were consistent with independent estimates derived from coded-wire tag returns.

Afin de distinguer les poissons porteurs de traits génétiques ou comportementaux qui peuvent être indésirables, nous avons examiné les écailles de saumon quinnat sauvage et d'élevage (*Oncorhynchus tshawytscha*) de la rivière Rakaia, en Nouvelle-Zélande, et d'un affluent, le cours d'eau Glenariffe. Les écailles de quinnat sauvage de type "eau douce" correspondaient à celles de juvéniles d'âge 1+, mais la taille moyenne des écailles de la zone correspondant à l'eau douce dans les écailles d'adultes de type "eau salée" était beaucoup plus importante que chez les juvéniles d'âge 0+. Les écailles de poissons d'élevage n'avaient pas de zone eau douce différente, mais on pouvait les distinguer des écailles de poissons sauvages par l'emplacement du premier anneau. L'analyse discriminante linéaire fondée sur l'emplacement du premier anneau a permis d'identifier correctement 82 à 90 % des poissons dans une population adulte mélangée, et de 88 à 92 % lorsque la longueur à la fourche et l'âge étaient inclus. Des estimations du nombre de poissons d'élevage pénétrant dans le cours d'eau Glenariffe entre 1989 et 1992, fondées sur l'analyse discriminante, étaient conformes aux estimations indépendantes découlant des retours des micromarques.

*Received February 17, 1993*
*Accepted May 21, 1993*
*(JB803)*

*Reçu le 17 février 1993*
*Accepté le 21 mai 1993*

Concern over detrimental impacts to wild (i.e. naturally spawned) salmonid populations from large-scale releases of hatchery fish has increased in recent years, with growing evidence of differences between salmonids of wild and hatchery origin. These include behavioural and morphological differences between wild and hatchery coho salmon (*Oncorhynchus kisutch*) (Taylor 1986; Swain and Riddell 1990; Swain et al. 1991) and genetically linked differences in growth and survival of steelhead trout (*Oncorhynchus mykiss*) (Reisenbichler and McIntyre 1977). While the management implications of these results may be debated (Hilborn 1992), there is acknowledgement that strategies for minimising possible adverse genetic interactions between wild and hatchery stocks need to be developed and implemented (Waples 1991). In New Zealand, high levels of straying (up to 38%) by hatchery-reared chinook salmon (*Oncorhynchus tshawytscha*) since 1978 (Unwin and Quinn 1993) have resulted in significant numbers of hatchery fish entering rivers up to 200 km from their natal catchment. The genetic status of New Zealand chinook stocks has not been studied, and it is unclear whether genetically distinct stocks have evolved since the first successful liberations in the early 1900s. However, there are significant interpopulation differences in life history traits such as age at maturity and length at age (Quinn and Unwin 1993), suggesting some degree of adaptation to specific rivers. Fishery managers concerned with natural production in these rivers therefore need to know what proportion of returning adults are of hatchery origin.

New Zealand chinook salmon hatcheries typically mark a proportion of their releases with coded-wire tags (CWTs) (Jefferts et al. 1963), and in principle, CWT recoveries can be used to estimate the contribution of hatchery fish. In practice, however, this approach often has serious limitations (Hankin 1982). In virtually all cases, calculations based on CWT data involve an "expansion factor" (Schnute 1992) to allow for the contribution from untagged hatchery fish and/or underreporting of recovered CWTs. Many New Zealand spawning streams are visited infrequently and the tag recovery rate (or "awareness factor") is low (e.g., 10–20%). In extreme cases, a single CWT

recovery may need to be multiplied by a total expansion factor of 100 or more, and the uncertainty in the estimated hatchery contribution may be comparable with the number of fish in the spawning population under study.

Several workers have explored the use of scale analysis, incorporating scale circulus and/or morphometric measurements, to distinguish between different populations of salmonids such as chinook salmon (Anas and Murai 1969; Major et al. 1973; Carl 1982) and sockeye salmon (*Oncorhynchus nerka*) (Cook 1982). Wild and hatchery chinook populations in New Zealand rivers should be amenable to this approach. Wild populations of chinook display two distinct patterns within the central portion (or nucleus) of the scale, corresponding to "ocean-type" and "stream-type" life histories (Gilbert 1913; Healey 1983; Flain and Glova 1988; Quinn and Unwin 1993). Ocean-type fish migrate to sea during the first year of life, at an age of 3–6 mo, while stream-type fish spend 12–15 mo in freshwater prior to seaward migration. By contrast, most hatchery chinook in New Zealand are released at ages 6–12 mo, during autumn and winter (Unwin et al. 1989, Unwin and Quinn 1993), giving rise to a distinctive "hatchery-type" scale (Flain 1991). Throughout this paper, we use the terms stream- and ocean-type to refer exclusively to chinook of wild origin.

The primary objectives of this study, based on analysis of scales from Rakaia River chinook salmon, were to characterise the scale patterns displayed by New Zealand chinook and to evaluate scale analysis as a method for estimating the proportion of hatchery fish in a mixed population. We proceeded in three stages. First, we examined the scale patterns displayed by adult and juvenile salmon of known origin, to describe (qualitatively and quantitatively) the features which characterise scales from ocean-, stream-, and hatchery-type fish, and to relate these features to freshwater residence patterns and/or hatchery release programmes. Second, we used linear discriminant function analysis (DFA) to classify fish by scale type, and hence to estimate the proportion of fish of hatchery origin in a mixed population. Third, we tested this estimation procedure by applying it to a spawning population (Glenariffe Stream) for which CWT data provided an independent estimate of the hatchery contribution.

## Methods

### Data Collection

To obtain data for chinook salmon populations of known origin, we examined scales from a variety of archival and current sources. The primary source was Glenariffe Stream, a headwater tributary of the Rakaia River, where an upstream counting fence has been maintained annually since 1965 (Flain 1982; Quinn and Unwin 1993). Hatchery facilities were constructed at Glenariffe in 1978, with up to 800 000 juvenile chinook salmon (including a proportion marked with CWTs and adipose-fin-clipped) released annually (Unwin et al. 1987, 1988b; Unwin 1991). Scales from chinook marked with CWTs were collected from returns to the hatchery and counting fence, sporadically from 1981 and routinely from 1989. Scales from tagged salmon also were collected from Coleridge Hatchery on the Rakaia River 13 km below Glenariffe, from annual fishing competitions on the Rakaia River (Quinn and Unwin 1993), from spawning ground surveys, and from commercial fishing vessels (Unwin et al. 1988a). Scales from juvenile hatchery salmon were collected from 127 groups of tagged fish, comprising 5000–15 000 fish marked with a unique CWT, released from Glenariffe between 1980 and 1991. For each CWT group, we randomly sampled 20 fish 1 d prior to their release.

Because of straying by hatchery chinook within the Rakaia catchment (Unwin and Quinn 1993), adult populations sampled since 1980 would have included fish of hatchery origin. To obtain data for scales from wild fish, we reexamined scales from 916 salmon caught by Rakaia anglers during creel surveys in 1973, 1974 and 1975 (West and Goode 1986), prior to the operation of any New Zealand hatchery. We also examined scales from 1243 juvenile salmon trapped on their downstream migration from Glenariffe Stream between October 1972 and October 1973 (Unwin 1986). Scales also were collected from 650 juvenile salmon caught in the Rakaia River during 1979, 1980, and 1981 (Hopkins and Unwin 1987), mostly from the lower reaches 60–100 km below Glenariffe Stream. These fish postdated the first hatchery releases from Glenariffe and would have included a few fish of hatchery origin; only one CWT was recovered (in September 1980) and we assumed the rest were of wild origin.

Commencing in 1989, we collected scales from a proportion of the unmarked fish entering Glenariffe Stream. All fish with an adipose fin clip were examined for the presence of CWTs. Since the entire spawning run is intercepted, this represents an awareness factor of 100% for the Glenariffe Stream spawning population.

### Preparation and Measurement of Scales

Scales were collected from the flank of the fish, posterior to the dorsal fin, and above the lateral line (Clutter and Whitesel 1956; Scarnecchia 1979). Scales collected prior to 1981 were mounted on glass microscope slides; those collected subsequently were stored as acetate impressions. Five scales from each fish were examined using a Nikon profile projector at 50×. Scale and circulus radii (as defined below) were measured from the scale focus along the anterior axis (Clutter and Whitesel 1956), using a Vernier micrometer, to the nearest 10 μm.

For scales from adult fish, the scale reader judged the position of the first annual check (Clutter and Whitesel 1956; Bilton 1975) and (where it could be clearly identified by an abrupt increase in mean circulus spacing) the end of the freshwater growth zone. We measured the radius ($R_1$) and number of circuli ($N_1$) to the first annual check and the corresponding parameters (denoted $R_f$ and $N_f$) for the zone of freshwater growth. Fish of wild origin were classified as ocean- or stream-type according to whether or not the zone of freshwater growth ended before or after the first annual check. We also measured $R_{15}$, the radius to the 15th circulus, because initial examination of scales from wild fish suggested that this might provide an objective datum, obtained without reference to the location of the first annulus, for differentiating between ocean- and stream-type fish. For scales from juvenile chinook, we determined the scale radius ($R_s$), the number of circuli ($N_s$), and (for age 1+ fish which clearly displayed an annual check) $R_1$ and $N_1$.

### Data Analysis

We computed mean circulus number and scale radius, both to the edge of the freshwater zone and to the first annulus, for all data sets. Results are expressed as the mean ± 1 SE unless otherwise stated. For adult fish, we used analysis of variance and post hoc *t*-tests to assess the significance of variations in mean circulus number and scale radius between years and age classes.

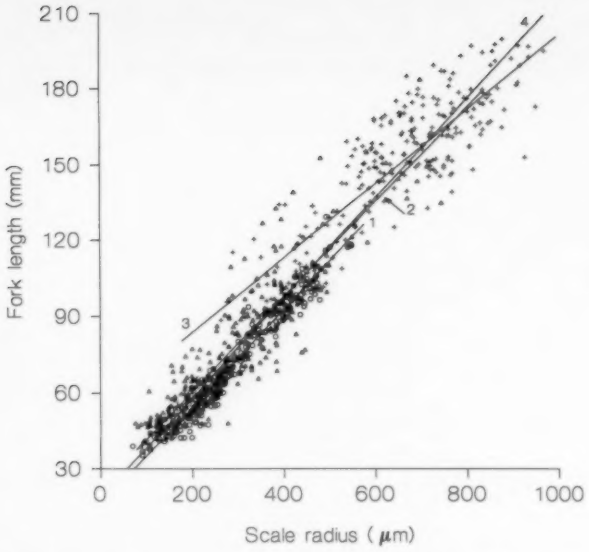



FIG. 1. Linear regressions of FL on scale radius for juvenile chinook salmon from Glenariffe Stream (○, 1, FL = 16.1 + 0.191$R_s$, $r^2$ = 0.971), the Rakaia River (△, 2, FL = 23.1 + 0.187$R_s$, $r^2$ = 0.832), and Glenariffe Hatchery (+, 3, FL = 54.1 + 0.147$R_s$, $r^2$ = 0.739). Line 4 is the regression equation for all data combined. A random sample of 300 points from each data set is shown.

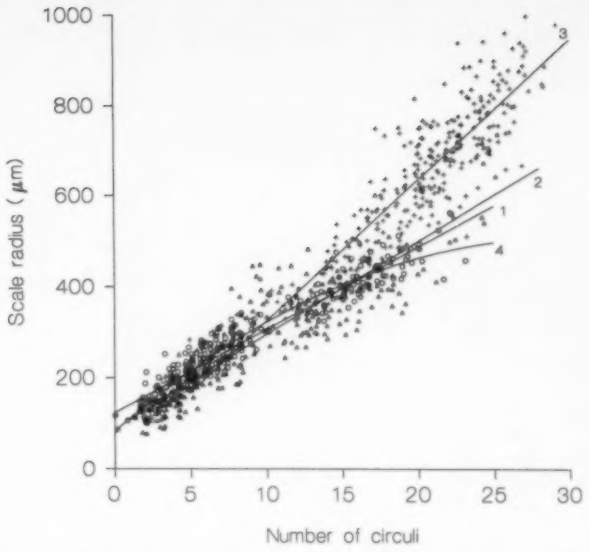



FIG. 2. Linear regressions of scale radius on number of circuli for juvenile chinook salmon from Glenariffe Stream (○, 1, $R_s$ = 124.0 + 18.2$N_s$, $r^2$ = 0.914), the Rakaia River (△, 2, $R_s$ = 90.0 + 20.5$N_s$, $r^2$ = 0.836), and Glenariffe Hatchery (+, 3, $R_s$ = 13.3 + 31.2$N_s$, $r^2$ = 0.799). Line 4 is a quadratic regression of scale radius on circulus number for the Glenariffe Stream data. A random sample of 300 points for each data set is shown.

For juvenile salmon, we constructed scatterplots and calculated linear regressions of fork length (FL) on scale radius for each data set and for all data combined (Fig. 1). We used the equation for all data (FL = 18.7 + 0.196$R_s$, $r^2$ = 0.934) to estimate the mean FL corresponding to the end of the freshwater zone, and to the deposition of the first annulus, for adults. We estimated mean circulus spacing (within the freshwater zone and/or to the first annulus) for adults by dividing the appropriate radius measurements, less the radius of the central platelet $R_0$ (i.e., the scale radius at a circulus number of zero), by the corresponding circulus number. Because $R_0$ measurements for fry with newly formed scales were not available for juveniles of Rakaia and hatchery origin, we estimated $\overline{R}_0$ for juveniles from Glenariffe Stream by constructing a quadratic regression of scale radius on circulus number (Fig. 2) to take account of a slightly nonlinear trend in the data for the smallest fry and interpreting the intercept on the vertical axis (84 ± 3 μm) as the desired estimate.

We used DFA to classify adult fish according to life history type, treating each year separately. We generated training data sets for each year, comprising equal numbers of fish with hatchery-, ocean-, and stream-type life histories, by combining data for all recovered fish marked with CWTs with data for equal numbers of ocean- and stream-type fish drawn at random from the 1973–75 samples. Discriminant functions were constructed using both the raw data and log transforms of scale radius and circulus number. To evaluate each set of discriminant functions, we cross-tabulated the DFA predictions against the known scale type for each fish and considered both the total percentage of fish classified correctly and the estimated proportion of hatchery fish compared with the theoretical value of 33.3%.

For the 1989–92 Glenariffe Stream spawning runs, we generated training data sets and constructed discriminant functions as described above, using data for tagged fish recovered at Glenariffe each year. We then used these functions to classify untagged fish, and hence to estimate the overall incidence of each life history type. Because the subsamples from which scales were taken were not necessarily a simple random sample of the total spawning population intercepted at the counting fence, and may have been biased with respect to FL (Flain 1982; Quinn and Unwin 1993), we estimated the proportion of fish of each life history type for the total population using a technique identical to the age–length key (Kimura 1977). We also estimated the overall incidence of hatchery fish by "expanding" the CWT return data to calculate the number of untagged hatchery fish present, 95% confidence limits being calculated using the parametric bootstrap method described by Geiger (1990).

## Results

### Ocean- versus Stream-Type Fish

Amongst wild adult chinook, scales from stream- and ocean-type fish were unambiguously distinguished by the location and dimensions of the first annulus (Table 1). Scales from stream-type fish averaged 15.7 ± 2.0 (range 11–22) circuli within the first year of life, compared with 33.1 ± 3.7 (range 23–45) for ocean-type fish. The radius to the first annulus overlapped very slightly (stream-type: 230 ≤ $R_1$ ≤ 680 μm; ocean-type 650 ≤ $R_1$ ≤ 1770 μm, but for practical purposes, measurements for the two life history types fell into two disjoint groups. For age 1+ fish of Glenariffe origin, the first annulus was clearly visible on scales from 28% (79 out of 284) of the fish examined. On most of these fish, the annulus appeared to form during September. Only 9% of the fish caught in August (14 out of 159) displayed a distinct annulus, but over half the fish caught in September and October (65 out of 125) did so.

TABLE 1. Scale measurements and back-calculated FLs (means ±1 SD) for Rakaia River adult and juvenile salmon of wild and hatchery origin. A dash indicates that a parameter could not be measured for, or did not apply to, the particular life history stage listed.

| Origin of sample | Edge of freshwater zone | | | First annulus | | Mean circulus spacing (μm) | | Mean/estimated mean fork length (mm) | |
|---|---|---|---|---|---|---|---|---|---|
| | Sample size | Number of circuli | Radius (μm) | Number of circuli | Radius (μm) | To edge of freshwater zone | To first annulus | At edge of freshwater zone | At first annulus |
| Glenariffe age 0+ | 950 | 5.8 ± 2.6 | 232 ± 63 | — | — | 25.5 ± 3.7 | — | 60 ± 12 | — |
| Rakaia age 0+ | 552 | 7.9 ± 4.4 | 255 ± 103 | — | — | 22.2 ± 5.7 | — | 69 ± 18 | — |
| Rakaia adults (ocean-type) | 541 | 9.4 ± 1.8 | 316 ± 61 | 33.1 ± 3.7 | 1090 ± 180 | 25.0 ± 4.8 | 30.3 ± 3.6 | 80 ± 11 | 231 ± 32 |
| Glenariffe age 1+ | 293 | 17.0 ± 2.4 | 427 ± 48 | 16.4 ± 1.9 | 410 ± 50 | 20.4 ± 2.2 | 20.0 ± 2.5 | 98 ± 10 | 95 ± 9 |
| Rakaia age 1+ | 98 | 18.2 ± 3.6 | 448 ± 121 | — | — | 19.9 ± 3.9 | — | 119 ± 21 | — |
| Rakaia adults (stream-type) | 375 | 16.8 ± 3.2 | 421 ± 86 | 15.7 ± 2.0 | 400 ± 60 | 20.0 ± 2.7 | 19.7 ± 2.7 | 107 ± 17 | 102 ± 12 |
| Hatchery juveniles | 1187 | 20.1 ± 4.4 | 646 ± 152 | — | — | 27.5 ± 3.7 | — | 150 ± 26 | — |
| Hatchery adults | 1520 | — | – | 24.8 ± 4.5 | 760 ± 180 | — | 26.8 ± 3.5 | — | 169 ± 34 |

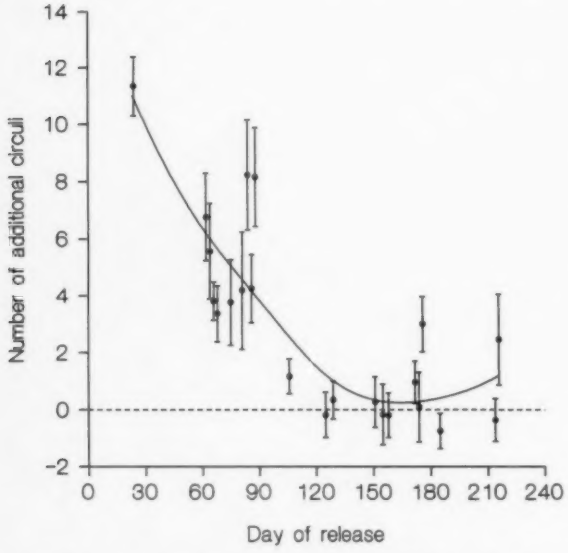



FIG. 3. Increase in mean number of circuli between the time of release and the end of the first year of life for 22 groups of chinook salmon marked with CWTs released from Glenariffe Hatchery, 1979–88, versus release date (day of year). Vertical bars indicate ±1 SE.

Mean first annulus radius for scales from adult fish showed some variation with age at maturity, but was generally stable between years. Amongst stream-type fish, neither $N_1$ nor $R_1$ varied from year to year (ANOVA, $p > 0.2$), although $R_1$ was on average slightly larger for 2-yr-old fish. However, the differences were small (age 2: $\overline{R}_1 = 414 \pm 15$ μm; ages 3–4: $\overline{R}_1 = 394 \pm 3$ μm; errors ± 1 SE) and barely significant (ANOVA, $p = 0.027$). For ocean-type fish, there was no variation between years (ANOVA, $p > 0.1$), but both $N_1$ and $R_1$ varied between age classes (ANOVA, $p < 0.0001$), being largest for 2-yr-olds. Mean values of $R_1$ (±1 SE) were age 2: 1204 ± 20 μm; age 3: 1075 ± 9 μm; age 4: 1018 ± 24 μm. Assuming that the relationship between juvenile FL and scale radius (Fig. 1) holds good up to scale radii of 1770 μm (the maximum value of $R_1$ recorded), these radii correspond to mean back-calculated FLs for ocean-type yearlings of 249, 228, and 219 mm for 2-, 3-, and 4-yr-old fish, respectively.

There was a strong correspondence between the dimensions of scales from age 1+ migrants and from stream-type adults (Table 1). Freshwater radius and circulus number for stream-type adults ($\overline{R}_f = 421$ μm; $\overline{N}_f = 16.8$) did not differ (ANOVA, $p > 0.2$) from the corresponding figures for age 1+ fish from Glenariffe Stream ($\overline{R}_s = 427$ μm; $\overline{N}_s = 17.0$). The freshwater zone on scales from Rakaia age 1+ fish tended to be more variable, being slightly larger ($\overline{R}_s = 448$ μm; $\overline{N}_s = 18.2$) than for either Glenariffe yearlings or stream-type adults. By contrast, the freshwater zone on scales from ocean-type adults ($\overline{R}_f = 316$ μm; $\overline{N}_f = 9.4$) was larger (ANOVA, $p < 0.001$) than scales from age 0+ fish in the Rakaia River ($\overline{R}_s = 255$ μm; $\overline{N}_s = 7.9$), which were in turn larger (ANOVA, $p < 0.001$) than scales from Glenariffe Stream age 0+ juveniles ($\overline{R}_s = 232$ μm; $\overline{N}_s = 5.8$).

## Hatchery versus Wild Fish

Scales from juveniles of hatchery origin were substantially larger than scales from wild juveniles (Fig. 1; Table 1), with an extensive band of widely spaced circuli. The mean number of circuli ($\overline{N}_1 = 20.1 \pm 4.4$) was only slightly higher than for wild yearlings (age 1+), but because of the increased circulus spacing (27.5 μm, compared with 19.9–20.4 μm for wild yearlings) the overall scale radius was approximately 50% larger. This characteristic also was apparent in scales from adult hatchery chinook, which tended to have a large central zone of very regular circuli, evenly and more widely spaced than for stream-type fish.

Mean circulus spacing for scales from hatchery adults (26.8 ± 3.5 μm) was not greatly different from mean circulus spacing in the freshwater and marine environments for ocean-type adults (25.0 ± 4.8 and 30.3 ± 3.6 μm, respectively). Consequently, we were often unable to locate the boundary of the freshwater zone in scales from hatchery adults. On many scales, the first annulus was the only significant feature within the first 30–40 circuli. We were unable to decide whether the edge of the freshwater zone could not be detected because of the relatively small increase in circulus spacing between the hatchery and marine environments, or whether it simply coincided with the first

TABLE 2. Interannual variation (means ± 1 SD) in the location of the first annulus for adult chinook of hatchery origin recovered in the Rakaia River, 1983–91.

| Year | Sample size | Circulus number | First annulus radius (μm) |
|---|---|---|---|
| 1983 | 4 | 20.5 ± 2.4 | 523 ± 78 |
| 1984 | 407 | 21.6 ± 3.3 | 652 ± 138 |
| 1985 | 67 | 20.5 ± 3.9 | 594 ± 134 |
| 1986 | 6 | 20.8 ± 4.2 | 592 ± 113 |
| 1987 | 13 | 22.0 ± 4.9 | 676 ± 201 |
| 1988 | 26 | 26.3 ± 3.7 | 797 ± 152 |
| 1989 | 95 | 27.2 ± 3.6 | 887 ± 140 |
| 1990 | 149 | 25.0 ± 3.9 | 783 ± 162 |
| 1991 | 173 | 25.0 ± 4.3 | 785 ± 182 |

TABLE 3. Accuracy of four linear discriminant functions used to classify adult Rakaia River chinook salmon, 1984–85 and 1989–91. Figures listed for each data set are the overall percentage of fish classified correctly and the discrepancy between the true incidence of each scale type (1:1:1) and the predicted incidence in terms of $\chi^2$. A $\chi^2$ value <5.99 indicates that the actual and predicted values did not differ significantly at a 95% confidence level.

| | | Parameters included in discriminant function(s) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $N_1$ | | $R_1$ | | $N_1, R_1$ | | $N_1, R_1$, FL, age | |
| Year | Number of hatchery scales available | % correct | $\chi^2$ | % correct | $\chi^2$ | % correct | $\chi^2$ | % correct | $\chi^2$ |
| 1984 | 407 | 88.6 | 6.38 | 87.0 | 11.2 | 89.8 | 12.0 | 92.2 | 3.45 |
| 1985 | 67 | 81.6 | 5.64 | 83.1 | 5.13 | 83.1 | 5.13 | 86.1 | 1.70 |
| 1989 | 95 | 84.6 | 0.53 | 81.8 | 0.44 | 85.3 | 0.15 | 88.1 | 0.34 |
| 1990 | 149 | 88.6 | 2.59 | 83.2 | 3.66 | 87.2 | 3.66 | 89.9 | 3.66 |
| 1991 | 173 | 89.4 | 1.70 | 85.9 | 1.35 | 89.0 | 1.54 | 91.9 | 1.16 |

annulus. By contrast, other hatchery scales displayed one or two distinct checks within the first annulus, typically between the 7th and 12th circuli and between the 15th and 25th circuli. The presence of these checks also confounded attempts to unambiguously locate the edge of the freshwater zone.

First annulus radius and circulus number for scales from hatchery adults ($\overline{R}_1 = 760$ μm; $\overline{N}_1 = 24.8$) were greater (ANOVA, $p < 0.001$) than mean scale radius and circulus number for hatchery juveniles ($\overline{R}_f = 646$ μm; $\overline{N}_f = 20.1$). We investigated growth patterns for hatchery fish, between the time of release and the end of the first year of life, by comparing adult and juvenile scale data for 22 CWT groups for which at least five adult scales were available. For each group, we calculated $\overline{N}_r$ (from the juvenile data) and $\overline{N}_1$ (from the adult data) and plotted the difference between these two values as a function of release date (Fig. 3). Releases up to the end of March showed significant additional growth prior to formation of the first annulus (equivalent to four to six circuli for nine releases in March), but little or no growth was apparent for 12 groups of fish released between 16 April and 3 August.

The location and dimensions of the first annulus for scales from adults of hatchery origin varied between years (ANOVA, $p < 0.001$; Table 2). For the five years from 1983 to 1987, $\overline{R}_1$ and $\overline{N}_1$ (607 μm and 21.1 circuli, respectively) were approximately 20% smaller than for the four years from 1988 to 1991 ($\overline{R}_1 = 813$ μm; $\overline{N}_1 = 25.9$). Consequently, scales from hatchery fish up to and including 1987 were sometimes difficult to distinguish from scales from stream-type fish whereas those collected since 1988 tended to be more easily confused with scales from ocean-type fish.

## Discriminant Function Analysis

Because of the high annual variation among scales from salmon of hatchery origin, we developed separate discriminant functions for each year for which sufficient data were available (1984–85 and 1989–91). Discriminant functions using log-transformed scale measurements generally did not perform as well as those based on the raw data and were not explored further.

For all five years examined, discriminant functions based solely on first annulus location correctly classified 81.6–89.4% of the fish in the corresponding training data set (Table 3). In general, $N_1$ gave more accurate results than $R_1$ in terms of the overall percentage of scales identified correctly, although (with the exception of 1984) both parameters were about equally successful with respect to estimating the overall incidence of each life history type. $N_1$ and $R_1$ together did not significantly improve the results and actually gave worse results in 1990 and 1991 than $N_1$ alone. Inclusion of $R_{15}$ did not improve classification accuracy. The most successful functions, incorporating $N_1$, $R_1$, FL, and age, classified 86.1–92.2% of the fish correctly and yielded estimates of the overall proportion of each life history type that did not differ significantly ($\chi^2 < 5.99$, df = 2) from the theoretical figure of 1:1:1. For these functions, most of the discrimination was provided by the first canonical factor, with relatively little separation along the axis of the second canonical factor (Fig. 4). This is consistent with the high level of

TABLE 4. Cross-classification for the four-parameter discriminant functions used to construct the last column of Table 3.

| Year | Actual scale type | Calculated scale type | | | Total (actual) |
|---|---|---|---|---|---|
| | | Stream | Hatchery | Ocean | |
| 1984 | Stream | 383 | 24 | 0 | 407 |
| | Hatchery | 50 | 346 | 11 | 407 |
| | Ocean | 0 | 10 | 397 | 407 |
| | Total (calculated) | 433 | 380 | 408 | 1221 |
| 1985 | Stream | 59 | 8 | 0 | 67 |
| | Hatchery | 15 | 49 | 3 | 67 |
| | Ocean | 0 | 2 | 65 | 67 |
| | Total (calculated) | 74 | 59 | 68 | 201 |
| 1989 | Stream | 94 | 1 | 0 | 95 |
| | Hatchery | 5 | 76 | 14 | 95 |
| | Ocean | 0 | 14 | 81 | 95 |
| | Total (calculated) | 99 | 91 | 95 | 285 |
| 1990 | Stream | 148 | 1 | 0 | 149 |
| | Hatchery | 17 | 118 | 14 | 149 |
| | Ocean | 0 | 13 | 136 | 149 |
| | Total (calculated) | 165 | 132 | 150 | 447 |
| 1991 | Stream | 172 | 1 | 0 | 173 |
| | Hatchery | 11 | 147 | 15 | 173 |
| | Ocean | 0 | 15 | 158 | 173 |
| | Total (calculated) | 183 | 163 | 173 | 519 |

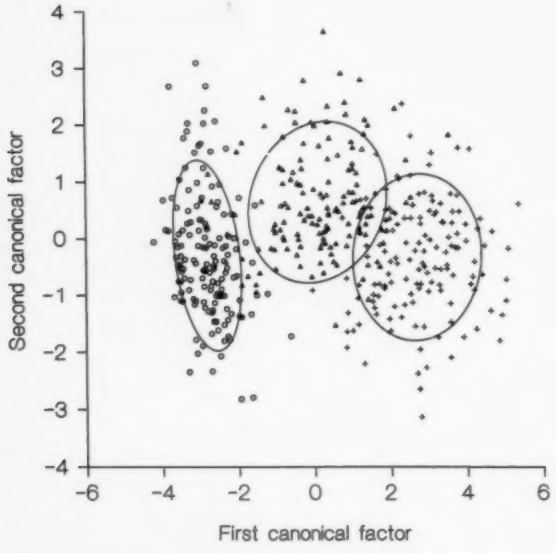



FIG. 4. Discriminant space scatterplot for a representative data set from Table 3 (1990, four-parameter discriminant function). ○, scales from stream-type fish; △, scales from hatchery fish; +, scales from ocean-type fish. Bivariate confidence ellipses enclosing ± 1 SD are also shown for each group.

discrimination provided by $N_1$ and/or $R_1$ alone, with the inclusion of additional parameters providing only a small increase in classification accuracy.

The cross-classification errors over the five years examined (Table 4) reflect the interannual variation in the characteristics of scales from salmon of hatchery origin (Table 2). For 1984 and 1985, the most common errors involved misclassification between hatchery- and stream-type fish whereas for 1989–91, errors involving hatchery- and ocean-type fish were more frequent. For the simplest discriminant functions (based on $N_1$), we compared variation between years by calculating decision rules in terms of the range of $N_1$ values for which an individual fish would be classified as of hatchery origin. These were 1984: $19 \leq N_1 \leq 27$; 1985: $19 \leq N_1 \leq 26$; 1989: $22 \leq N_1 \leq 30$; 1990 and 1991: $21 \leq N_1 \leq 29$.

## Classification of Glenariffe Stream Fish

To classify Glenariffe Stream fish, all four parameters listed in Table 3 were used to derive discriminant functions for each year. The estimated proportion of hatchery fish was relatively constant from year to year, ranging from 66.0 to 76.6% (Table 5), despite a twofold variation in the total spawning population. Fish of wild origin were predominantly ocean-type. Considering just the wild component of each run, the estimated proportion of ocean-type fish ranged from 75.6% (in 1992) to 89.7% (in 1989).

With the exception of 1989, the incidence of hatchery fish estimated from scale data and from CWT returns was in good agreement (Table 5), although confidence intervals for the CWT estimates were relatively broad. For 1989, the CWT estimate of 2234 fish (89.7% of the total run of 2492 fish) was substantially higher than the scale estimate of 1645 (66.0%). One factor which may have contributed to this discrepancy was the arrival, in Glenariffe Stream, of a substantial number of salmon from the

TABLE 5. Incidence of wild stream-type, wild ocean-type, and hatchery-origin fish entering Glenariffe Stream, 1989–92, estimated by DFA of scale data, together with the estimated incidence of hatchery fish based on expanded CWT returns. Confidence intervals (±95%) for the CWT estimates were generated by a bootstrap simulation of 5000 samples, as described in the text.

| Year | Total number of fish | Estimated incidence of fish according to scale type (DFA) | | | Number of tagged fish | Estimated incidence of hatchery fish (CWT) | |
|---|---|---|---|---|---|---|---|
| | | Wild (stream) | Wild (ocean) | Hatchery | | Number | % incidence |
| 1989 | 2492 | 82 (3.3%) | 765 (30.7%) | 1645 (66.0%) | 155 | 2234 (1705–2492) | 89.7 (68.4–100) |
| 1990 | 3056 | 143 (4.7%) | 645 (21.1%) | 2268 (74.2%) | 129 | 2074 (1658–2506) | 67.9 (54.3–82.0) |
| 1991 | 1861 | 81 (4.4%) | 343 (18.4%) | 1426 (76.6%) | 157 | 1289 (1057–1546) | 69.3 (56.8–83.1) |
| 1992 | 1592 | 113 (7.1%) | 350 (22.0%) | 1129 (70.9%) | 154 | 1156 (927–1421) | 72.6 (58.2–89.3) |

Tentburn Hatchery, a large commercial hatchery on the coast 10 km north of the Rakaia River mouth. CWTs of Tentburn origin were recorded from 14 (9.0%) of the 155 tagged fish recovered, including two from release groups with expansion factors as high as 113:1. Although scale measurements for these 14 fish did not differ significantly from those for fish originating from Glenariffe Hatchery, a larger sample ($N = 148$) of fish returning to Tentburn in 1989 had comparatively broad first annuli ($\overline{R}_1 = 981 \pm 132$ μm; $\overline{N}_1 = 31.7 \pm 3.1$; $N = 148$), only slightly smaller than for ocean-type fish. Because of the high expansion factor for the Tentburn CWTs, fish of Tentburn origin contributed disproportionately to the estimated total of 2234 hatchery fish (708 fish, or 31.7%) but were underrepresented in the data used to derive the 1989 discriminant functions. Therefore, it is possible that scales collected from a more representative sample of hatchery fish — including a higher proportion of Tentburn origin — would have yielded a higher estimate of the overall incidence of hatchery fish.

## Discussion

### Scale-Type Characteristics

The general appearance of scales from stream-type and ocean-type adult chinook from the Rakaia River, up to and including the first annulus, was consistent with the life history patterns of age 1+ and 0+ juvenile migrants, respectively. For stream-type adults, the freshwater zone was composed of a compact band of closely spaced circuli, corresponding almost exactly in mean radius and number of circuli to scales from yearling juveniles in both Glenariffe Stream and the Rakaia River. Scales from ocean-type adults were characterised by a smaller freshwater zone, consistent with a residence period of 3–6 mo. However, the relatively large mean freshwater radius for ocean-type adults represents a mean FL of 80 mm at seawater entry, 10–20 mm greater than the mean FL of age 0+ juveniles from the Rakaia River and Glenariffe Stream. While some of this discrepancy may be attributable to higher mortality rates for smaller fish, this result is consistent with the findings of Unwin (1981, 1986) and Hopkins and Unwin (1987) that outmigrant juvenile chinook from the lower Rakaia River enter the ocean at about 80–90 mm FL and that age 0+ migrants from Glenariffe Stream spend additional time rearing in the Rakaia mainstem in order to reach this size.

As well as being larger, scales from Rakaia underyearlings also were more variable (in terms of the correlations between $R_1$ and FL, and $N_1$ and $R_1$) than scales from Glenariffe underyearlings. We attribute this in part to the greater variability of the Rakaia River environment, and the aggregation of data from a number of sampling locations, in contrast with the much more stable Glenariffe Stream environment (Unwin 1986) within which data were collected from a single site. However, some of the variation may reflect the composite nature of the Rakaia population, which comprises fish from three major spawning grounds other than Glenariffe Stream (West and Goode 1987). Significant phenotypic differences exist between adult chinook from Glenariffe Stream and at least one of these spawning grounds (Quinn and Unwin 1993). The decrease in mean first annulus radius and back-calculated FL with increasing age at maturity, for ocean-type fish, is consistent with the general tendency for age at maturity in salmonids to be inversely correlated with juvenile growth rate (Thorpe 1986 and references therein).

Circulus spacing is positively correlated with growth rate in several fish species (Glenn and Mathias 1985; Doyle et al. 1987), including coho salmon (Fisher and Pearcy 1990) and sockeye salmon (Bilton 1975). Therefore, the general appearance of scales from hatchery chinook, with large nuclei and widely spaced circuli, can be directly attributed to increased growth rates within the hatchery environment. Multiple checks within the freshwater zone of some hatchery-type scales also are consistent with hatchery rearing conditions. Multiple checks in hatchery populations of juvenile sockeye have been linked to sudden changes in growth rate, such as those induced by feeding fish to satiation after an extended period of reduced growth (Bilton and Robins 1971; Bilton 1975). Glenariffe chinook are normally fed on demand (Unwin et al. 1989), but disruption to this regime (e.g., by tagging or grading) could result in an interrupted growth pattern conducive to the formation of ancillary checks. In a feeding trial at Glenariffe Hatchery in autumn 1992, two experimental groups of juvenile chinook were fed a reduced diet for 3 mo, fed to satiation for 4 wk, returned to the reduced diet for a further 3 mo, and finally returned to full demand feeding. From scale samples taken at monthly intervals, we observed one additional check on 95.4% of the scales examined after the first introduction of demand feeding, two checks on 89.6% of the scales examined after the second period of reduced feeding, and three checks on 68.0% of the scales sampled at the end of the trial.

Amongst fisheries managers in New Zealand, there is debate over whether chinook released from upstream hatcheries such as

Glenariffe undergo a significant amount of instream rearing before entering the ocean (McDowall 1991). Our results indicate a dependence on time of release. For late autumn and winter releases (April–August), no additional circuli were laid down prior to formation of the first annulus, indicating that little if any instream rearing occurred. This is consistent with results for outmigrant chinook from Glenariffe Stream (Unwin 1986), showing little or no increase in mean FL from May to August. For late summer and early autumn releases (January–March), some additional rearing obviously occurred during the first year of life, but we were unable to determine from the scales alone whether this took place in the freshwater or marine environment. Unwin et al. (1989) noted that most chinook released from Glenariffe appeared to pass through the lower Rakaia River within 48 h of their release, although some fish from a March release were caught, by seining in backwaters within the lower 15 km of the river, over 5 wk later. A few fish from February and April liberations were recaptured in the lagoon at the mouth of the Rakaia River up to 151 d after their release (Eldon and Greager 1983). For releases prior to April, therefore, some additional freshwater rearing may occur during the remainder of the first year of life.

We interpret the increased size of scales from hatchery chinook during the 1980s as a reflection of increased growth rates resulting from improvements in hatchery practices at Glenariffe (Unwin et al. 1989). These include the introduction of demand feeding (in 1983), routine grading of all stock (since 1985), and accelerated incubation of ova at Silverstream Hatchery (on a tributary of the lower Waimakariri River) where water temperatures are 3–5°C warmer than in Glenariffe Stream (Hardy 1988).

### Stock Identification

The generally high level of agreement between the DFA and expanded CWT estimates of the incidence of hatchery fish at Glenariffe is encouraging. Although cross-classification arrays can be used to gauge the accuracy of DFA in relation to the particular data set from which the discriminant functions were derived, we are unaware of any other studies where independent validation has been possible. It is perhaps unfortunate that for all four years of record, the estimated proportion of hatchery fish entering Glenariffe Stream was in the order of 70%, so that it was not possible to test DFA on a population in which wild fish predominated.

Based on a detailed analysis of returns to hatcheries in British Columbia, Schnute et al. (1990) reported that expanded CWT data provided estimates of the number of salmon returning to their hatchery of origin that were, on average, 22% lower than figures provided by direct counts. Notwithstanding the rather broad confidence intervals (typically ± 15%) for our expanded CWT estimates, our data show no evidence of any systematic discrepancy of this magnitude.

The accuracy with which we were able to classify wild and hatchery chinook, using relatively simple scale measurements (82–89% for single-parameter discriminant functions and 86–92% for four-parameter functions), compares favourably with results from other studies. Using three scale parameters, Carl (1982) correctly identified the origin of 66% of a mixed population of wild and hatchery juvenile chinook from Lake Michigan. A simple decision rule based on only one scale parameter correctly identified approximately 85% of the fish in a mixed stock of hatchery and wild steelhead trout (Seelbach and Whelan 1988). More recently, proprietary software and digitising equipment have been used to measure a range of scale parameters for wild and hatchery barramundi (*Lates calcarifer*) (Barlow and Gregg 1991), and striped bass (*Morone saxatilis*) (Ross and Pickard 1990), yielding classification accuracies of up to 83 and 87–91%, respectively.

In all of the above studies, wild fish comprised a single discrete population. For Rakaia chinook, the need to distinguish between two types of wild fish complicates the analysis slightly, since hatchery scales can be confused with either large stream-type scales or small ocean-type scales. In this respect, our results may be compared with those from several studies on the use of scale patterns to classify stocks according to river, region, or continent of origin. For example, Major et al. (1973) correctly classified 72–88% of Asian and western Alaskan chinook stocks (from up to five Alaskan subareas) as to continent of origin, and Cook and Lord (1978) correctly classified 77% of the fish from three age 2.2 Bristol Bay sockeye populations. More recent studies (using a microcomputer to process scale luminance data) have yielded classification accuracies of 35–52% for samples of five Fraser River sockeye stocks (Cook and Guthrie 1987). These latter figures are relatively low, but are significant given the rather subtle differences between most of the Fraser populations. Image processing techniques, taking into account additional parameters such as scale shape (Ross and Pickard 1990), offer some potential for further studies on New Zealand chinook, although we consider it unlikely that these would yield classification accuracies much in excess of 90%.

Possible misclassification errors resulting from interannual variation between scale characters for individual stocks need to be considered when applying DFA to data sets of unknown origin (Major et al. 1973; Bilton and Messinger 1975; Cook and Guthrie 1987). For Rakaia chinook, we detected no significant interannual variation between scales from ocean- and stream-type fish, although given the variation in first annulus location with age at maturity, some interannual variation could arise if a particular year class was significantly underrepresented. Incorporating age at maturity into DFA should help to accommodate any such variation. A more critical assumption in our study is that the characteristics of scales from stream- and ocean-type chinook during the period 1984–92 were the same as during 1973–75. While we have no reason to believe otherwise, the absence of a suitable sample of wild fish for the latter period precludes an independent test of this assumption. We encountered significant interannual variation in the parameters of hatchery-type scales, leaving us with little alternative but to derive separate discriminant functions for each year. However, first annulus measurements for 1990 and 1991 were very similar, suggesting that annual variation may be decreasing as hatchery technology stablises.

Our results confirm earlier reports of a significant decline in the number of wild fish returning to Glenariffe Stream (Unwin 1991). The average return for the years 1989–92, 616 fish (using the DFA estimates) or 562 (using the CWT data), represents only one third of the 1965–78 average of 1849 fish (Flain 1982). Increasingly, concerns about the possible detrimental effects of large-scale releases of hatchery fish on the continued viability of wild populations are being expressed by fishery managers throughout the world (Ryman and Stahl 1981; Waples 1991; Hilborn 1992). In New Zealand, there is an urgent need to determine the incidence of hatchery fish in the major east coast chinook salmon rivers in order to develop rational policies for managing the fishery.

## Acknowledgements

We thank Tom Gough, Malcolm Flain, Selwyn Hawke, Martin Tawa, and Julian Sykes for technical assistance, David Baird for advice on statistical techniques, and John Kelso, Bob McDowall, John Hayes, and two anonymous referees for critically reviewing an early draft of the manuscript. This research was funded in part by the Foundation for Research, Science and Technology, through grant CO 1203.

## References

ANAS, R.E., AND S. MURAI. 1969. Use of scale characters and a discriminant function for classifying sockeye salmon (*Oncorhynchus nerka*) by continent of origin. Int. North Pac. Fish. Comm. Bull. 26: 157–192.

BARLOW, C.G., AND B.A. GREGG. 1991. Use of circuli spacing on scales to discriminate hatchery and wild barramundi, *Lates calcarifer* (Bloch). Aquacult. Fish. Manage. 22: 491–498.

BILTON, H.T. 1975. Factors influencing the formation of scale characters. Int. North Pac. Fish. Comm. Bull. 32: 102–108.

BILTON, H.T., AND H.B. MESSINGER. 1975. Identification of major British Columbia and Alaska runs of age 1.2 and 1.3 sockeye from their scale characters. Int. North Pac. Fish. Comm. Bull. 32: 109–129.

BILTON, H.T., AND G.L. ROBINS. 1971. Effects of feeding level on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 28: 861–868.

CARL, L.M. 1982. Scale parameters of wild and hatchery salmon (*Oncorhynchus* tshawytscha) from Lake Michigan. Can. Tech. Rep. Fish. Aquat. Sci. 1105: 19 p.

CLUTTER, R.I., AND L.E. WHITESEL. 1956. Collection and interpretation of sockeye salmon scales. Bull. Int. Pac. Salmon Fish. Comm. 9: 159 p.

COOK, R.C. 1982. Stock identification of sockeye salmon (*Oncorhynchus nerka*) with scale pattern recognition. Can. J. Fish. Aquat. Sci. 39: 611–617.

COOK, R.C., AND I. GUTHRIE. 1987. In-season stock identification of sockeye salmon (*Oncorhynchus nerka*) using scale pattern recognition, p. 327–334. *In* H.D. Smith, L. Margolis, and C.C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

COOK, R.C., AND G.E. LORD. 1978. Identification of stocks of Bristol Bay sockeye salmon, *Oncorhynchus nerka*, by evaluating scale patterns with a polynomial discriminant method. Fish. Bull. 76: 415–423.

DOYLE, R.W., A.J. TALBOT, AND R.R. NICHOLAS. 1987. Statistical interrelation of length, growth, and scale circulus spacing: appraisal of a growth rate estimator for fish. Can. J. Fish. Aquat. Sci. 44: 1520–1528.

ELDON, G.A., AND A.J. GREAGER. 1983. Fishes of the Rakaia Lagoon. N.Z. Minist. Agric. Fish. Fish. Environ. Rep. 30: 65 p.

FISHER, J.P., AND W.G. PEARCY. 1990. Spacing of scale circuli versus growth rate in young coho salmon. Fish. Bull. 88: 637–643.

FLAIN, M. 1982. Quinnat salmon runs, 1965-1978, in the Glenariffe Stream, Rakaia River, New Zealand. N.Z. Minist. Agric. Fish. Fish. Div. Occas. Publ. 28: 22 p.

FLAIN, M. 1991. The state of the wild salmon fishery, p. 15–17. *In* M.J. Unwin, S.F. Davis, and M.C. Hall [ed.] Salmon towards 2000: Proceedings of a conference held at Addington Raceway, Christchurch, 27–28 October 1990. N.Z. Minist. Agric. Fish. Freshwater Fish. Rep. 129.

FLAIN, M., AND G.J. GLOVA. 1988. A test of the reliability of otolith and scale readings of chinook salmon (*Oncorhynchus tshawytscha*). N.Z. J. Mar. Freshwater Res. 22: 497–500.

GEIGER, H.J. 1990. Parametric bootstrap confidence intervals for estimating contributions to fisheries from marked salmon populations. Am. Fish. Soc. Symp. 7: 667–676.

GILBERT, C.H. 1913. Age at maturity of the Pacific coast salmon of the genus *Oncorhynchus*. Bull. U.S. Bur. Fish. 32: 1–23.

GLENN, C.L., AND J.A. MATHIAS. 1985. Circuli development on body scales of young pond-reared walleye (*Stizostedion vitreum*). Can. J. Zool. 63: 912–915.

HANKIN, D.G. 1982. Estimating escapement of Pacific salmon: marking practices to discriminate wild and hatchery fish. Trans. Am. Fish. Soc. 111: 286–298.

HARDY, C.J. 1988. Establishment and operation of the Silverstream fish hatchery, 1964 to 1982. N.Z. Minist. Agric. Fish. Freshwater Fish. Rep. 102: 132 p.

HEALEY, M.C. 1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type chinook salmon, *Oncorhynchus tshawytscha*. Can. Field-Nat. 97: 427–433.

HILBORN, R. 1992. Hatcheries and the future of salmon in the northwest. Fisheries 17: 5–8.

HOPKINS, C.L., AND M.J. UNWIN. 1987. River residence of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, South Island, New Zealand, N.Z. J. Mar. Freshwater Res. 21: 163–174.

JEFFERTS, K.B., P.K. BERGMAN, AND H.F. FISCUS. 1963. A coded-wire identification system for macro-organisms. Nature (Lond.) 198: 460–462.

KIMURA, D.K. 1977. Statistical assessment of the age-length key. J. Fish. Res. Board Can. 34: 317–324.

MAJOR, R.L., S. MURAI, AND J. LYONS. 1973. Scale studies to identify Asian and western Alaskan chinook salmon. Int. North Pac. Fish. Comm. Annu. Rep.: 80–91.

MCDOWALL, R.M. 1991. When is enough enough — or — what don't we know about salmon that we need to know?, p. 50–55. *In* M.J. Unwin, S.F. Davis, and M.C. Hall [ed.] Salmon towards 2000: Proceedings of a conference held at Addington Raceway, Christchurch, 27–28 October 1990. N.Z. Minist. Agric. Fish. Freshwater Fish. Rep. 129.

QUINN, T.P., AND M.J. UNWIN. 1993. Variation in life history patterns among New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. Can. J. Fish. Aquat. Sci. 50: 1414–1421.

REISENBICHLER, R.R., AND J.D. MCINTYRE. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 34: 123–128.

ROSS, W.R., AND A. PICKARD. 1990. Use of scale patterns and shape as discriminators between wild and hatchery striped bass stocks in California. Am. Fish. Soc. Symp. 7: 71–77.

RYMAN, N., AND G. STAHL. 1981. Genetic perspectives of the identification and conservation of Scandinavian stocks of fish. Can. J. Fish. Aquat. Sci. 38: 1562–1575.

SCARNECCHIA, D. 1979. Variation of scale characteristics of coho salmon with sampling location on the body. Prog. Fish-Cult. 41: 132–135.

SCHNUTE, J.T. 1992. Statistical analysis of embedded replicates in mark-recovery experiments. Can. J. Fish. Aquat. Sci. 49: 432–442.

SCHNUTE, J.T., T.J. MULLIGAN, AND B.R. KUHN. 1990. An errors-in-variable bias model with an application to salmon hatchery data. Can. J. Fish. Aquat. Sci. 47: 1453–1467.

SEELBACH, P.W., AND G.E. WHELAN. 1988. Identification and contribution of wild and hatchery steelhead stocks in Lake Michigan tributaries. Trans. Am. Fish. Soc. 117: 444–451.

SWAIN, D.P., AND B.E. RIDDELL. 1990. Variation in agonistic behaviour between newly emerged juveniles from wild and hatchery populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 47: 566–571.

SWAIN, D.P., B.E. RIDDELL, AND C.B. MURRAY. 1991. Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. Can. J. Fish. Aquat. Sci. 48: 1783–1791.

TAYLOR, E.B. 1986. Differences in morphology between wild and hatchery populations of juvenile coho salmon. Prog. Fish-Cult. 48: 171–176.

THORPE, J.E. 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting, p. 7–14. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

UNWIN, M.J. 1981. Aspects of the juvenile quinnat salmon outmigration from the Glenariffe Stream, p. 15–20. *In* C.L. Hopkins [ed.] Proceedings of the Salmon Symposium. N.Z. Minist. Agric. Fish. Fish. Res. Div. Occas. Publ. 30.

UNWIN, M.J. 1986. Stream residence time, size characteristics, and migration patterns of juvenile chinook salmon (*Oncorhynchus tshawytscha*) from a tributary of the Rakaia River, New Zealand, N.Z. J. Mar. Freshwater Res. 20: 231–252.

UNWIN, M.J. 1991. Hatchery releases and the salmon sports fishery, p. 18–25. *In* M.J. Unwin, S.F. Davis, and M.C. Hall [ed.] Salmon towards 2000: Proceedings of a conference held at Addington Raceway, Christchurch, 27–28 October 1990. N.Z. Minist. Agric. Fish. Freshwater Fish. Rep. 129.

UNWIN, M.J., R.A. DOUGHERTY, AND P.R. TODD. 1988a. Investigations into the catching of salmon by commercial fishing vessels. N.Z. Minist. Agric. Fish., Freshwater Fish. Rep. 100: 43 p.

UNWIN, M.J., M.S. FIELD-DODGSON, D.H. LUCAS, AND S.P. HAWKE. 1989. Experimental releases of coded-wire tagged juvenile chinook salmon (*Oncorhynchus tshawytscha*) from the Glenariffe Salmon Research Station, 1982–83 to 1984–85. N.Z. Minist. Agric. Fish. Fish. Tech. Rep. 10: 22 p.

UNWIN, M.J., D.H. LUCAS, AND T. GOUGH. 1987. Coded-wire tagging of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand, 1977–86. N.Z. Minist. Agric. Fish. Fish. Tech. Rep. 2: 24 p.

UNWIN, M.J., D.H. LUCAS, AND T. GOUGH. 1988b. Coded-wire tagging of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand, 1977–81: release programmes and returns at maturity. N.Z. Minist. Agric. Fish. Fish. Data Rep. 33: 50 p.

UNWIN, M.J., AND T.P. QUINN. 1993. Homing and straying patterns of chinook salmon (*Oncorhynchus tshawytscha*) from a New Zealand hatchery: spatial distribution of strays and effects of release date. Can. J. Fish. Aquat. Sci. 50: 1168–1175.

WAPLES, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific northwest. Can. J. Fish. Aquat. Sci. 48: 124–133.

WEST, I.F., AND R.H. GOODE. 1986. Postal surveys of anglers fishing for sea-run chinook salmon on the Rakaia River, Canterbury, New Zealand, 1973/74 and 1974/75. N.Z. J. Mar. Freshwater Res. 20: 345–354.

WEST, I.F., AND R.H. GOODE. 1987. Aerial counts of spawning chinook salmon (*Oncorhynchus tshawytscha*) on the Rakaia River system, Canterbury, New Zealand, 1973-76. N.Z. J. Mar. Freshwater Res. 21: 563–572.

# Estimation of Trap Selectivity for Male Snow Crab (*Chionoecetes opilio*) Using the SELECT Modeling Approach with Unequal Sampling Effort

Xucai Xu[1]

*Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada*

and Russell B. Millar

*Department of Mathematics and Statistics, University of Otago, P.O. Box 56, Dunedin, New Zealand*

Xu, X., and R.B. Millar. 1993. Estimation of trap selectivity for male snow crab (*Chionoecetes opilio*) using the SELECT modeling approach with unequal sampling effort. Can. J. Fish. Aquat. Sci. 50: 2485–2490.

An experiment was conducted to study trap selectivity for male snow crab (*Chionoecetes opilio*) in St. Mary's Bay, Newfoundland. The finishing effort of large-mesh commercial traps and small-mesh control traps was not equal because two commercial traps were fished for every control trap. We show how the SELECT (Share Each Length's Catch Total) method extends to this situation and, more generally, to selectivity trials in which catches are sampled in unequal proportion. Application to the crab data showed that a good fit was provided by the logistic selection curve under the assumption of unequal fishing efficiencies of commercial and control traps. The carapace widths at the retention probabilities of 0.25, 0.50, and 0.75 were estimated to be 86.4, 94.6, and 102.9 mm, respectively, and the retention probability at the minimum legal size of 95 mm was estimated to be 0.51. The commercial traps had almost twice the fishing efficiency of the control traps and consequently caught more legal-sized crabs per trap.

Une expérience a été menée pour étudier la sélectivité des casiers pour la capture de crabe des neiges mâles (*Chionoecetes opilio*) dans la baie St. Mary's, à Terre-Neuve. L'effort de pêche des casiers de pêche commerciale de gros maillage et des casiers témoins de petit maillage n'était pas égal parce que deux casiers de pêche commerciale étaient mouillés pour chaque casier témoin. Nous montrons comment la méthode SELECT (*Share Each Length's Catch Total*) s'étend à cette situation, et, plus généralement, à des essais de sélectivité dans le cadres desquels les captures sont échantillonnées en proportion inégale. L'application aux données sur le crabe a montré qu'un bon ajustement était assuré par la courbe de sélectivité logistique en vertu de l'hypothèse selon laquelle les casiers de pêche commerciale et les casiers témoins ne possèdent pas la même efficacité de pêche. Les largeurs de carapace pour des probabilités de conservation de 0,25, 0.50, et 0,75 ont été estimées respectivement à 86,4, 94,6 et 102,9 mm, et la probabilité de conservation à la taille minimale légale de 95 mm a été évaluée à 0,51. L'efficacité pêche des casiers de pêche commerciale était presque le double de celle des casiers témoins et permettait donc de capturer plus de crabes de taille légale par casier.

*Received December 11, 1992*
*Accepted June 4, 1993*
*(JB722)*

*Reçu le 11 décembre 1992*
*Accepté le 4 juin 1993*

Japanese-style, conical, baited traps with a stretched-mesh size of 133 mm are used for commercial fishing of male snow crab (*Chionoecetes opilio*) in Newfoundland. This mesh size was voluntarily used by many fishermen in the early 1980's because smaller meshed traps caught more noncommercial crabs and increased the sorting time (D. Taylor, Department of Fisheries and Oceans, Science Branch, St. John's, Nfld., personal communication). Shortly thereafter 133 mm was regulated as the minimum mesh size.

Catches of male crabs off the southeast coast of Newfoundland were quantitatively studied by Miller (1976) using traps of three different mesh sizes (91, 119 and 129 mm stretched-mesh). Hoenig and Dawe (1991) compared catches in small-mesh (25 mm) traps, commercial traps, and trawl gear. These studies looked only at the differences in relative frequency of size classes in the various gears and did not attempt estimation of selection curves. Both studies noted that the number of noncommercial-sized crabs decreased with increasing trap mesh size. In addition, Miller (1976) observed that the numbers of large (≥102 mm carapace width) crabs were not significantly different between the three mesh sizes used. Hoenig and Dawe (1991) found that the commercial trap caught significantly more legal-sized (≥95 mm carapace width) crabs than the small-mesh trap.

A higher catch of legal-sized crabs in the commercial trap than the small-mesh trap implies that escapement, if any, of legal-sized crabs from the commercial trap is more than offset by increased entry. Here we used SELECT (Millar 1992; Millar and Walsh 1992) to quantify these concepts in terms of the selection curve and relative fishing efficiency (with respect to a small-mesh trap) of the commercial trap. SELECT is a rigorous statistical modeling approach that has been developed to analyse data from selectivity experiments using comparative catches from two or more gears. Millar and Walsh (1992) demonstrated

[1]Present address: Department of Mathematics and Statistics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

its applicability to catch data from trouser trawl or alternate haul experiments and showed how it models unequal fishing efficiencies of the gears deployed.

The method was dubbed SELECT (Share Each Length's Catch Total) because it fits to the share of the total catch of each size class that is taken by each gear, rather than fitting to ratios of catches (e.g., Pope et al. 1975). This avoids the problems of dividing by zero or having a "proportion retained" that exceeds unity. Fonteyne (1991) analysed twin trawl data using SELECT and a traditional estimator, remarking that SELECT should be preferred because it does not manipulate the data.

Applications of the SELECT modeling technique to trouser trawl experiments include Suuronen et al. (1991), Suuronen and Millar (1992), and Walsh et al. (1992). Cadigan and Millar (1992) used simulation to show that SELECT estimators are "well behaved" (have predictable behaviour) and have better performance than previous traditional estimators. Millar (1992) showed that SELECT can also be applied to gillnet and hook selectivity experiments.

The application of SELECT presented here extends the method to cases in which the experimental and control gears are not fished with equal effort, or are not sampled in equal proportion. The latter situation is common in trawl selectivity trials.

## Materials and Methods

### Experimental Design

Preseason (August 23 to September 2) and postseason (October 21–31) research surveys were carried out in 1991 in St. Mary's Bay, Newfoundland, using conical-shaped, Japanese-style, top-entry traps baited with squid. The two surveys had 40 and 41 sets, respectively, randomly located in the crab habitat areas of St. Mary's Bay in water deeper than 40 m. Two 25-mm-mesh traps (control gear) and four 133-mm-mesh commercial traps (experimental gear) were used in each set. Unsuccessful hauls due to bait loss or overturning were eliminated, reducing the total numbers of successful and commercial trap and control trap hauls to 305 and 155, respectively.

The commercial traps had a 122-cm diameter base tapering to a 71-cm-diameter top. The trap frame, made from six iron rods welded together, was covered with 133-mm (inside stretched length) diamond polyethylene mesh with a funnel-shaped plastic cone at the top to allow crabs to enter but preventing their escape. The control traps were of identical construction except that they were covered with 25-mm mesh. Each of the six traps was attached by a 3.7-m-long support rope (1.3 cm in diameter) to the groundline (1.6 cm in diameter) at intervals of 37 m. A 14-kg weight was attached to each end of the groundline to inhibit shifting and overturning of the end traps. The buoyline, made of the same rope as the groundline, was 20–30 fathoms longer than the depth of the water to allow for current, wind, and depth changes. An inflatable buoy and high-flier (radar reflector buoy) were used on each end of the buoyline. Soak time (duration of immersion) was approximately 24 h, subject to the weather. A more detailed description of gear deployment is found in Dawe (1989).

The size–frequency distribution of the catch by trap type was constructed by measuring the crab carapace widths to the nearest millimetre and grouping by 5-mm intervals (Table 1).

### Selection Curves and SELECT Modeling

The plastic cone under the trap entrance effectively eliminates escape by this route (Miller 1990). Since escapement is entirely through the trap mesh, it is reasonable to postulate that the selection curve will be similar to that for trawls. The most commonly used trawl selection curve is the symmetric logistic function (Pope et al. 1975)

$$(1)\quad r(l) = \frac{\exp(a + bl)}{1 + \exp(a + bl)}$$

where $r(l)$ is the probability that an animal of size $l$ will be retained and parameters $a$ and $b$ are to be estimated. Since $r(l)$ will tend to 1 as size increases, and will tend to 0 as size approaches 0, we deduce that $a < 0$ and $b > 0$.

The adequacy of the logistic selection curve can be formally tested by fitting a more general curve. For example, to check for possible asymmetry of the selection curve (Millar 1991), a selection curve of the form

$$(2)\quad r(l) = \left(\frac{\exp(a + bl)}{1 + \exp(a + bl)}\right)^{1/\delta}$$

can be fitted. Parameter $\delta$ quantifies the amount of asymmetry, and a check of symmetry is given by a statistical test of the hypothesis $H_\delta : \delta = 1$.

SELECT includes a "split" parameter, $p$, to quantify any possible difference in the intensities with which data from the experimental and control gears are collected. The factors contributing to the relative intensities of data collection are (i) relative fishing efforts, (ii) relative fishing efficiencies, and (iii) relative sampling efforts. In most comparative selectivity experiments the gears are fished with equal effort. However, this is not the case here because there were 305 successful commercial trap hauls compared with 155 with the control traps. Denoting these relative fishing efforts by $f_1$ and $f_2$, respectively, and assuming for convenience that they sum to unity gives $f_1 = 305/460$ and $f_2 = 155/460$. Relative fishing efficiencies (denoted by $c_1$ and $c_2$, respectively) can be considered the relative probabilities of entry given contact with the gear. To be consistent with Millar (1992) and Millar and Walsh (1992), it is assumed for convenience that $c_1 + c_2 = 1$. Millar (1992) called the combined effects of factors (i) and (ii) the relative fishing intensities. Relative sampling efforts ($s_1$ and $s_2$, respectively) are the proportions of the catches that are measured. In this present study, $s_1$ and $s_2$ are both unity because all crabs were measured. It is not uncommon for these sampling proportions to be small (e.g., <0.1) in trawl selectivity studies in which catches are large. It will be assumed that $f_1, f_2, s_1$, and $s_2$ are known.

The following conditional probabilities are required to apply the SELECT argument (Millar 1992; Millar and Walsh 1992). The probability that a size $l$ animal is measured, given that it encountered the selectivity gear, is

$$P(\text{measured} \mid \text{encounter})$$
$$= P(\text{measured from gear 1} \mid \text{encounter}) + P(\text{measured from gear 2} \mid \text{encounter}).$$

For $i = 1$ or 2,

$$(3)\quad P(\text{measured from gear } i \mid \text{encounter})$$
$$= P(\text{measured from gear } i \mid \text{caught in gear } i) \times P(\text{caught in}$$

TABLE 1. Numbers of male snow crab caught in the preseason (August 23 to September 2, 1991) and postseason (October 21–31, 1991) research surveys using Japanese-style, conical, baited traps in St. Mary's Bay, Newfoundland.

| Carapace width class (mm) | Preseason survey | | Postseason survey | | Total | |
|---|---|---|---|---|---|---|
| | Large mesh ($n$ = 148) | Small mesh ($n$ = 76) | Large mesh ($n$ = 157) | Small mesh ($n$ = 79) | Large mesh ($n$ = 305) | Small mesh ($n$ = 155) |
| 26–30 | 0 | 0 | 0 | 1 | 0 | 1 |
| 31–35 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36–40 | 0 | 1 | 0 | 0 | 0 | 1 |
| 41–45 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46–50 | 0 | 3 | 0 | 0 | 0 | 3 |
| 51–55 | 2 | 22 | 0 | 9 | 2 | 31 |
| 56–60 | 1 | 78 | 2 | 58 | 3 | 136 |
| 61–65 | 12 | 221 | 15 | 214 | 27 | 435 |
| 66–70 | 39 | 395 | 65 | 422 | 104 | 817 |
| 71–75 | 78 | 518 | 114 | 561 | 192 | 1079 |
| 76–80 | 164 | 547 | 227 | 699 | 391 | 1246 |
| 81–85 | 462 | 785 | 670 | 922 | 1132 | 1707 |
| 86–90 | 1239 | 1160 | 1388 | 1277 | 2627 | 2437 |
| 91–95 | 1880 | 1049 | 1923 | 1102 | 3803 | 2151 |
| 96–100 | 1360 | 649 | 1256 | 526 | 2616 | 1175 |
| 101–105 | 922 | 391 | 812 | 276 | 1734 | 667 |
| 106–110 | 644 | 211 | 587 | 177 | 1231 | 388 |
| 111–115 | 442 | 128 | 424 | 90 | 866 | 218 |
| 116–120 | 178 | 40 | 172 | 36 | 350 | 76 |
| 121–125 | 43 | 9 | 38 | 15 | 81 | 24 |
| 126–130 | 9 | 2 | 9 | 2 | 18 | 4 |
| 131–135 | 1 | 0 | 1 | 0 | 2 | 0 |
| Total | 7476 | 6209 | 7703 | 6387 | 15179 | 12596 |
| Avg. per trap | 50.5 | 81.7 | 49.1 | 80.8 | 49.8 | 81.3 |
| Total ≥95 mm | 3958 | 1616 | 3665 | 1328 | 7623 | 2944 |
| Avg. per trap | 26.7 | 21.3 | 23.2 | 16.8 | 25.0 | 19.0 |

$$\text{gear } i \mid \text{enter gear } i) \times P(\text{enter gear } i \mid \text{encounter gear } i) \times P(\text{encounter gear } i \mid \text{encounter})$$
$$= k s_i r_i(l)\, c_i f_i$$

where $r_i(l)$ is the selection curve of gear $i$. The constant of proportionality, $k$, is due to $c_1$ and $c_2$ being *relative* probabilities.

Let the experimental and control gears be gears 1 and 2, respectively. The relative fishing efforts will be denoted by $f$ and $1 - f$, respectively, and the gear efficiencies by $c$ and $1 - c$, respectively. It will be assumed that the control gear retains all animals (i.e., $r_2(l) = 1$) and the selection curve of the experimental gear will be denoted $r(l)$. Then, the proportion of the total number (in experimental and control gears) of measured size $l$ animals that is from the experimental gear has expected value (Millar and Walsh 1992)

$$\phi(l) = \frac{P(\text{measured from experimental gear} \mid \text{encounter})}{P(\text{measured} \mid \text{encounter})}$$

which is from equation (3)

$$(4)\quad \phi(l) = \frac{s_1 r(l) cf}{s_1 r(l)\, cf + s_2(1-c)(1-f)} = \frac{\dfrac{qcr(l)}{1-c}}{\dfrac{qcr(l)}{1-c} + 1}$$

where $q = s_1 f / s_2 (1-f)$ is known. The parameters to be estimated in equation (4) are $c$ and the parameters of the selection curve, $r(l)$.

Existing SELECT software (Millar and Cadigan 1991; Millar 1993a; R.B. Millar, undocumented *Splus* code) uses maximum likelihood, under the assumption that the observed proportions are binomially distributed, to fit

$$(5)\quad \phi(l) = \frac{pr(l)}{pr(l) + (1-p)} = \frac{\dfrac{pr(l)}{1-p}}{\dfrac{pr(l)}{1-p} + 1}$$

to the observed proportions of measured size $l$ animals that are from the experimental gear. Estimation of the parameters in equation (5) is equivalent to estimation of the parameters in equation (4) because the two equations are the same when we set

$$\frac{qc}{1-c} = \frac{p}{1-p}$$

which gives

$$(6)\quad c = \frac{p}{q + (1-q)\, p}.$$

Equivalently, for any value of $c$,

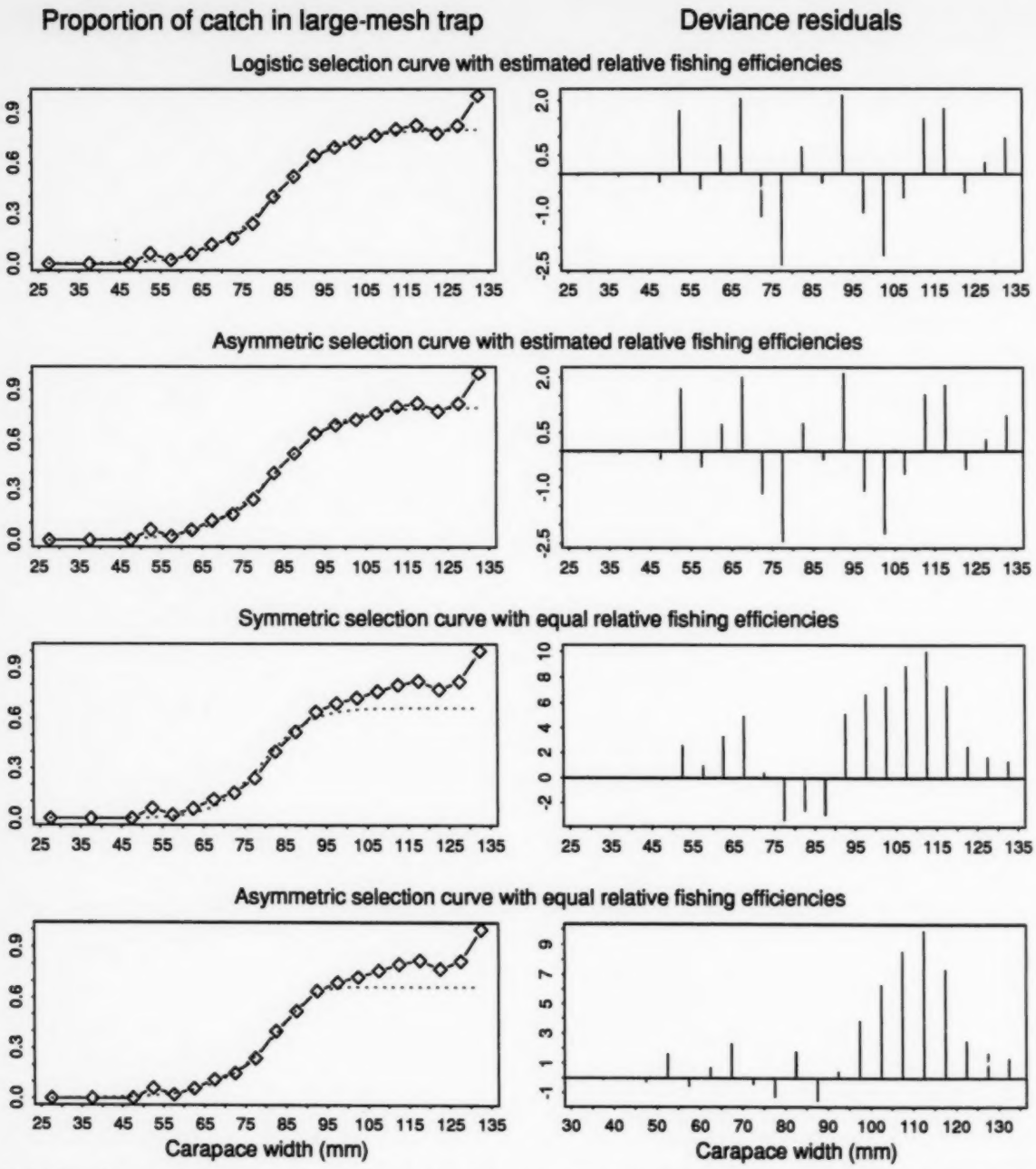

Proportion of catch in large-mesh trap
Deviance residuals
Logistic selection curve with estimated relative fishing efficiencies
Asymmetric selection curve with estimated relative fishing efficiencies
Symmetric selection curve with equal relative fishing efficiencies
Asymmetric selection curve with equal relative fishing efficiencies
Carapace width (mm)
Carapace width (mm)

FIG. 1. Fits to the proportion of total male snow crab catch taken by the commercial (133-mm mesh) traps, and the associated deviance residuals.

$$(7)\quad p = \frac{qc}{1 + (q - 1)\,c}.$$

In this study, we used the SELECT modeling approach to fit equation (5) with $r(l)$ defined by equation (1) or (2). Since $q$ = 305/155, the hypothesis of equal fishing efficiency, $H_c : c = 0.5$, is equivalent to the hypothesis $H_p : p = 305/460$ by equation (7). The fits were done with $p$ fixed at the value 305/460, and also with $p$ being estimated, so that comparison of the four models ($p$ fixed/estimated; logistic/asymmetric selection curve) would permit testing of hypotheses $H_p$ and $H_\delta$. Note that the simplest model (logistic selection curve and $p$ = 305/460) requires estimation of only parameters $a$ and $b$ whereas the most complicated model (asymmetric selection curve and estimated $p$) requires estimation of $a$, $b$, $p$, and $\delta$.

TABLE 2. Estimates of the parameters from fits of the logistic and asymmetric selection curves for the equal fishing efficiency ($p = 305/460$) and estimated relative fishing efficiency cases. The selection factor is the length of 50% retention ($l_{50}$) divided by mesh size (133 mm). The selection range is $l_{75}$–$l_{25}$. Values in parentheses are 95% percentile confidence intervals obtained from bootstrapping individual sets.

| | Logistic selection curve | | Asymmetric selection curve | |
|---|---|---|---|---|
| | $p = 305/460$ | Estimated $p$ | $p = 305/460$ | Estimated $p$ |
| $a$ | −15.5 | −12.6 (−14.0–−11.3) | −52.4 | −12.8 |
| $b$ | 0.182 | 0.133 (0.114–0.150) | 0.570 | 0.135 |
| $p$ | 305/460 | 0.795 (0.762–0.829) | 305/460 | 0.794 |
| $\delta$ | 1 | 1 | 4.67 | 1.02 |
| $l_{25}$ | 79.3 | 86.4 (84.2–89.2) | 80.6 | 86.3 |
| $l_{50}$ | 85.4 | 94.6 (91.7–98.6) | 86.3 | 94.5 |
| $l_{75}$ | 91.4 | 102.9 (99.5–107.9) | 90.1 | 102.9 |
| Relative fishing efficiency ($c$) | 0.5 | 1.97 (1.62–2.46) | 0.5 | 1.96 |
| Selection factor (SF) | 0.642 | 0.711 (0.688–0.743) | 0.649 | 0.711 |
| Selection range (SR) | 12.1 | 16.5 (14.5–19.3) | 9.5 | 16.4 |
| SR/$l_{50}$ | 0.142 | 0.174 (0.153–0.195) | 0.110 | 0.17 |
| Model deviance | 432.2 | 32.9 | 303.1 | 32.9 |
| Degrees of freedom | 18 | 17 | 17 | 16 |

## Results

Examination of the residuals from the fits of equation (5) (to the observed proportions of total catch taken in the large-mesh traps) shows that the equal fishing efficiency hypothesis ($H_p$ : $p = 305/460$) is untenable (Fig. 1). In comparison, the residuals from both fits with estimated relative fishing efficiencies show no obvious structure (Fig. 1). There is little apparent difference between the logistic and asymmetric selection curve fits when relative fishing efficiencies are estimated.

Inspection of the model deviances (the likelihood ratio statistic for model goodness of fit) shows that the asymmetric selection curve provides no improvement (to one decimal place) to the estimated $p$ fits (Table 2). That is, the hypothesis of symmetry ($H_\delta : \delta = 1$) was not rejected and the logistic selection curve with unequal relative fishing efficiencies is therefore the best model (Fig. 2). This model estimates the carapace widths of 25, 50, and 75% reduction ($l_{25}$, $l_{50}$, and $l_{75}$) to be 86.4, 94.6, and 102.9 mm, respectively. The selection factor (SF = $l_{50}$ /mesh size) is 94.6/133.0 = 0.711.

The deviance of the above model is 32.9 on 17 degrees of freedom, indicating a statistically significant ($p$-value = 0.01) amount of overdispersion (McCullagh and Nelder 1989). A crude estimate of overdispersion is provided by dividing model deviance by degrees of freedom. When catch data from individual sets are available, then it is better to use the nonparametric replication estimate of dispersion (McCullagh and Nelder 1989, p. 127; Suuronen and Millar 1992), or to formally model between haul variation (Fryer 1991), or to bootstrap (Millar 1993b) if the computational power is available. Here, the latter approach was taken with the 81 individual sets being resampled with replacement. As described in Millar (1993b), the individual animals are also resampled within each chosen set. For each of 200 bootstraps performed, the logistic selection curve was fitted under the assumption of unequal fishing efficiency. Residual plots from all 200 bootstraps were used to check that the fits were satisfactory. Confidence intervals for the selectivity parameter estimates (Table 2) were obtained using the percentile method (Efron 1982, p. 78).

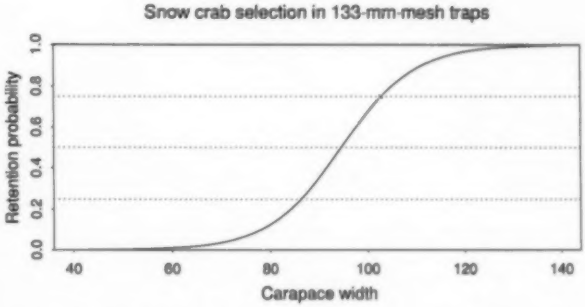



FIG. 2. Logistic selection curve for male snow crab in commercial (133-mm mesh) traps.

Parameter $p$ was estimated to be 0.795. That is, 79.5% of crabs entered large-mesh traps, although these traps made up only 305/406 = 66.3% of the total used. Conversely, 20.5% of entry

was into small-mesh traps, although they constituted 33.7% of the total used. Therefore, the relative fishing efficiency of large-mesh to small-mesh traps was 79.5/66.3:20.5/33.7, or 1.97:1. Scaling these relative fishing efficiencies to sum to unity produces the values given by equation (6), $c = 0.663$ and $1 - c = 0.337$.

## Discussion

The small-mesh traps caught more crabs (81.3 per trap) than the commercial traps (49.8 per trap) but caught fewer legal-sized crabs (19.0 per small-mesh trap compared with 25.0 per large-mesh trap) despite the retention probability of the smallest legal-sized (95 mm) crab in the commercial trap being only 51%, estimated from equation (1). This was because of a higher fishing efficiency of the commercial traps — SELECT estimated that approximately two (1.97) crabs entered a commercial trap for every crab entering a small-mesh trap.

A likely explanation for the higher fishing efficiency of the commercial traps is that, because of the considerable escapement of small- and medium-sized crabs, many more crabs can enter these traps before trap saturation begins limiting entry. Miller (1979) studied trap saturation of the red crab (*Cancer productus*) and Dungeness crab (*Cancer magister*) by looking at the difference in catch of "fished" traps (crabs were removed from the traps every 2 h) and "not-fished" traps (traps lifted every 2 h, but no crabs removed). He found that the catch in "fished" traps was significantly higher after 4 h and increased to a ratio of 2.7:1 after 12 h. However, in his recent review paper, Miller (1990) cautioned that "The popular view that catch per trap of commercial sizes increases if more subcommercial animals are permitted to escape was not confirmed by several of the studies reviewed. . ." and added that this could be due to inadequate experimental design or by limited escapement of commercial-sized animals. In this present case, the catch of legal-sized crabs was greater in the commercial traps because the difference in fishing efficiency more than compensated for some escapement.

As with trawl surveys, selectivity and relative efficiencies will vary with fishing strategy. In particular, the relative fishing efficiencies of commercial and control traps will depend greatly on trap saturation and therefore on soak time and abundance of crab. For these reasons, the research survey data used here were gathered under conditions (e.g., bait used, soak time) similar to those of the commercial fishery. We felt that this was far preferable to the alternative experimental procedure of placing a measured population of crabs into large-mesh traps and recording those remaining in the traps after a given soak time. The alternative design will affect the behaviour of the crabs and does not include the dynamics of the processes of entry and escapement. In such an experiment with American lobster (*Homarus americanus*) (Krouse 1978), retention probability was seen to increase from 0.2 to 1.0 with just a 2-mm increase in carapace width. Under commercial conditions, the entry of crabs into and escapement of crabs from the trap are gradual (subject to gear saturation and bait life) and selectivity would not be so sharp. Here, the selection range ($l_{75} - l_{25}$) was estimated to be 16.5 mm.

This study has demonstrated that logistic selection curves can provide a satisfactory description of escapement from traps. This claim is based on the SELECT fit, and therefore implicitly assumes that the relative fishing efficiencies of the commercial and control traps are independent of crab size. Intimidation of smaller crabs, especially as the traps fill, could lead to a violation of this assumption. It is not possible to test this assumption using the data of this experiment, since size variability in relative fishing efficiencies is confounded with size selectivity. Miller (1976) found that the proportion of subcommercial snow crabs decreased slightly with increased soak time. He considered the effect to be negligible and felt that it was due to the smaller crabs having a longer time in which to attempt escape.

## Acknowledgements

We thank John Hoenig, Earl Dawe, and David Taylor for helpful discussions and comments and Earl Dawe, Paul Beck, Joe Drew, and Gerry Dawe for their help in collecting the data.

## References

CADIGAN, N.G., AND R.B. MILLAR. 1992. The reliability of selection curves obtained from trouser trawl or alternate haul experiments. Can. J. Fish. Aquat. Sci. 49: 1624–1632.

DAWE, E. 1989. Exploratory crab survey Labrador, 1989. Project Report FDD 1989-147: iv + 29 p.

EFRON, B. 1982. The jackknife, the bootstrap and other resampling plans. SIAM Monogr. No. 38, CBSM-NSF.

FONTEYNE, R. 1991. Comparison between the covered condend method and the twin trawl method in beam trawl selectivity experiments. ICES C.M. 1991/B:52: 26 p.

FRYER, R.J. 1991. A model of between-haul variation in selectivity. ICES J. Mar. Sci. 48: 281–290.

HOENIG, J.M., AND E.G. DAWE. 1991. Relative selectivity of four sampling methods using traps and trawls for male snow crabs (*Chionoecetes opilio*). CAFSAC Res. Doc. 91/32: 18 p.

KROUSE, J.S. 1978. Effectiveness of escape vent shape in traps for catching legal-sized lobster, *Homarus americanus*, and harvestable-sized crabs, *Cancer borealis* and *Cancer irroratus*. Fish. Bull. 76: 425–432.

MCCULLAGH, P., AND J.A. NELDER. 1989. Generalized linear models. 2nd ed. Chapman and Hall, New York, N.Y. 511 p.

MILLAR, R.B. 1991. Estimation of asymmetric selection curves for trawls. ICES C.M. 1991/B:56: 21 p.

MILLAR, R.B. 1992. Estimating the size-selectivity of fishing gear by conditioning on the total catch. J. Am. Stat. Assoc. 87: 962–968.

MILLAR, R.B. 1993a. Analysis of trawl selectivity studies (addendum): implementation in SAS. Fish. Res. 17: 373–377.

MILLAR, R.B. 1993b. Incorporating between-haul variation using bootstrapping and nonparametric estimation of selection curves. Fish. Bull. 91: 564–572.

MILLAR, R.B., AND N.G. CADIGAN. 1991. A FORTRAN program for fitting selectivity curves to trouser trawl data. Can. Tech. Rep. Fish. Aquat. Sci. 1783: iii + 19 p.

MILLAR, R.B., AND S.J. WALSH. 1992. Analysis of trawl selectivity studies with an application to trouser trawls. Fish. Res. 13: 205–220.

MILLER, R.J. 1976. Trap mesh selection and catches of the spider crab (*Chionoecetes opilio*). Fish. Mar. Serv. Tech. Rep. 598: iv + 15 p.

MILLER, R.J. 1979. Saturation of crab traps: reduced entry and escapement. J. Cons. Int. Explor. Mer 38(3): 338–345.

MILLER, R.J. 1990. Effectiveness of crab and lobster traps. Can. J. Fish. Aquat. Sci. 47: 1228–1251.

POPE, J.A., A.R. MARGETTS, J.M. HAMLEY, AND E.F. AKYUZ. 1975. Manual of methods for fish stock assessment, Part III. Selectivity of fishing gear. FAO Fish. Tech. Pap. 41: 65 p.

SUURONEN, P., AND R.B. MILLAR. 1992. Size selectivity of diamond and square mesh codends in pelagic herring trawls: only small herring will notice the difference. Can. J. Fish. Aquat. Sci. 49: 2104–2117.

SUURONEN, P., R.B. MILLAR, AND A. JARVIK. 1991. Selectivity of diamond and hexagonal mesh codends in pelagic herring trawls: evidence of a catch size effect. Finn. Fish. Res. 12: 143–156.

WALSH, S.J., R.B. MILLAR, C.G. COOPER, AND W.M. HICKEY. 1992. Codend selection in American plaice: diamond versus square mesh. Fish. Res. 13: 235–254.

# Population Field Theory with Applications to Tag Analysis and Fishery Modeling: the Empirical Green Function

Carlos A.M. Salvadó

*Southwest Fisheries Science Center, NMFS, NOAA, P.O. Box 271, La Jolla, CA 92038, USA*

Salvadó, C.A.M. 1993. Population field theory with applications to tag analysis and fishery modeling: the empirical Green function. Can. J. Fish. Aquat. Sci. 50: 2491–2512.

A theoretical framework is proposed for analyzing fish movement and modeling the associated dynamics using tagging data. When tagged fish are released in an area small compared with the domain of the fish population and over a period short compared with the time they take to disperse throughout their domain, the pattern of movement approximates a point-source solution of the underlying population dynamics. A method of point sources (Green functions) is invoked for representing the solution of the tagged and untagged fish field equations (partial differential equations) in terms of integral equations. As an approximate representation of a tagging experiment, the Green function is interpreted as the probability density of survival and movement from point to point in space–time. The Green functions are constructed empirically using one parameter, catchability, as the ratio of population density of tagged fish divided by the number of tagged fish released. The number of tagging experiments necessary to characterize the population is dictated by the dependence of catchability on space–time. The moments of the Green function are used to calculate model parameters and lead to the identification of a closed form expression for the transition probability densities of the model assumed.

Un plan de travail théorique est proposé pour analyser les déplacements des poissons et modéliser la dynamique connexe au moyen de données de marquage. Lorsque des poissons marqués sont relâchés dans une zone de faibles dimensions par rapport au domaine occupée par la population de poissons et sur une courte période par rapport au temps de dispersion nécessaire dans leur domaine, le profil de déplacement correspond à peu près à une solution de source ponctuelle de la dynamique des populations sous-jacente. Une méthode de sources ponctuelles (fonction de Green) est retenue pour représenter la solution des équations de mouvement des poissons marqués et non marqués (équations aux dérivées partielles) en fonction d'équation intégrales. À titre de représentation approximative d'une expérience de marquage, la fonction de Green est interprétée comme la densité de probabilité de la survie et du déplacement d'un point à l'autre dans l'espace et dans le temps. Les fonctions de Green sont établies de façon empirique à l'aide d'un paramètre, la vulnérabilité, qui correspond au rapport entre la densité de population des poissons marqués divisée par le nombre de poissons marqués relâchés. Le nombre d'expériences de marquage nécessaires pour caractériser la population est dicté par la dépendance de la vulnérabilité à l'égard du temps et de l'espace. Les moments de la fonction de Green sont utilisés pour calculer les paramètres du modèle et permettent l'établissement d'une expression fermée des densités de la probabilité de transition du modèle hypothétique.

*Received September 24, 1991*
*Accepted June 21, 1993*
*(JB232)*

*Reçu le 24 septembre 1991*
*Accepté le 21 juin 1993*

## 1. Introduction

Considerable effort has been devoted to understanding animal movement patterns (Okubo 1980; Murray 1989). In particular, the study of fish movement patterns by the use of tagging experiments has been reviewed by Hilborn (1990), who discussed recent studies on the subject (Bayliff 1979; Ishii 1979; Sibert 1984; Hunter et al. 1986; Kleiber et al. 1987). These studies require the assumption of a model (a partial differential equation, or, in general, a field equation) parameterized in such ways so that it accommodates the movement of tagged fish and the catch and natural death rate densities. The model parameters are typically evaluated by means of the tagging data and such extreme value principles as maximum likelihood estimators.

A field equation is also employed in the empirical method discussed here. It is one of great generality that can be stated simply as the inhomogeneous advection–diffusion equation (Okubo 1980) with mortality for dilute populations with spatially and temporally varying coefficients. The empirical method that will be exposed in this work is a one-parameter model and is adequate for answering the question on fisheries interaction posed in Section 2. It is also the first step that would be taken to prepare the data for fitting a multiple parameter model.

In a typical tagging experiment, tagged fish are released over a time interval short in relation to the time they disperse throughout their domain and into an area small in relation to the domain of the population. Under these conditions, the release and recovery data from the experiment approximate a point-source solution of the field equations that represent the population dynamics of the fish. The method of solving inhomogeneous linear differential equations by use of their point-source solutions has been known for many years, and the subject is referred to as the method of Green functions (Feynman 1949; Courant and Hilbert 1953; Morse and Feshbach 1953; Bjorken and Drell

1964; Garabedian 1964; Feynman and Hibbs 1965; Byron and Fuller 1970; Gilbarg and Trudinger 1979). The Green function associated with a linear differential equation is the solution of the differential equation under the influence of a unit impulsive source at one point (i.e., a source of unit strength applied at one space–time point) and therefore is the point-source solution.

In general, if tagged fish were released at every space–time point, the subsequent recapture results would differ between points owing to uneven distributions of fishing effort in space–time. In addition, there may be other influences that affect the patterns of the point sources such as geographic (e.g., boundaries) and cyclic factors (e.g., seasonal, El Niño). Nevertheless, each of the release and recovery measures still represents a point-source solution of the field equations that describe the underlying fish dynamics and, therefore, in some manner must be approximately a Green function associated with the dynamics.

Apparently, Green functions have never been used in modeling tagged fish populations, the reason probably being that the use of the method of Green functions requires linearity, and fish population dynamics processes, such as recruitment, are thought to be nonlinear. By separating the linear and the nonlinear parts, and by considering the nonlinear portion as being part of the term that makes the field equation inhomogeneous, the method of Green functions becomes not only useful, but conceptually simple, in fish population modeling.

The following symbols are used:

$\mathcal{A}$ = domain of the population
$\mathbf{R}^2$ = infinite plane
$\partial\mathcal{A}$ = boundary of $\mathcal{A}$
$\mathbf{n}_\perp$ = unit vector normal to $\partial\mathcal{A}$ pointing outward
$\mathbf{R}$ = infinite line
$\partial\mathcal{A}_A$ = portion of $\partial\mathcal{A}$ on which population is absorbed
$\partial\mathcal{A}_R$ = portion of $\partial\mathcal{A}$ from which population is reflected
$\mathcal{A}_i$ = neighborhood of point $\mathbf{r}_i$ referred to as a cell
$n$ = number of nonoverlapping cells defined in $\mathcal{A}$
$\mathbf{i}, \mathbf{j}$ = unit vectors respectively in the $+x$ and $+y$ directions
$\{O_i: i = 1, 2, \ldots\}$ = set whose members are $O_1, O_2 \ldots$
$\in$ = member of
$\subseteq$ = subset or equal to
$\cup$ = union
$<$ = less than
$>$ = greater than
$\ll$ = much less than
$\gg$ = much greater than
$\leq$ = less than or equal to
$\geq$ = greater than or equal to
$\sim$ = similar to, of the same order of magnitude
$\mathbf{r} = x\mathbf{i} + y\mathbf{j}$ = position vector $\in \mathcal{A}$

$r = \sqrt{\mathbf{r} \cdot \mathbf{r}}$ = magnitude of vector $\mathbf{r}$
$\mathbf{r}_T = x_T\mathbf{i} + y_T\mathbf{j}$ = location of tag release $\in \mathcal{A}$
$t$ = time
$a$ = age
$t_T$ = time of tag release
$[t_i, t_f] = t_f - t_i$
$\mathcal{T}_l = [t_l, t]$
$\mathcal{T}_T = [t_T, t] \subseteq \mathcal{T}_l$
$\mathcal{T}_j = [t_j, t_{j+1}]$
$\mathcal{T}' = [t', t]$
$\mathcal{T}$ = interval of time of arbitrary length; a period
$m$ = number of intervals of time $\mathcal{T}_j$ into which $\mathcal{T}_l$ is subdivided

$e(\mathbf{r},t)$ = fishing effort density field
$c_T(\mathbf{r},t)$ = tagged fish catch density rate field
$c(\mathbf{r},t)$ = untagged fish catch density rate field
$p_T(\mathbf{r},t)$ = tagged fish population density field
$p(\mathbf{r},t)$ = untagged fish population density field
$dl$ = infinitesimal line element
$d^2\mathbf{r}$ = infinitesimal surface element = $dxdy$ in Cartesian coordinates
$\nabla$ = differential operator $\mathbf{i}\partial/\partial x + \mathbf{j}\partial/\partial y$
$\triangle = \nabla \cdot \nabla = \partial^2/\partial x^2 + \partial^2/\partial y^2$
$\mathbf{j}(\mathbf{r},t)$ = flux of $p$
$s(\mathbf{r},t)$ = source and/or sink density rate of field $p$
$d(\mathbf{r},t)$ = natural death density rate field
$m(\mathbf{r},t)$, $m(t)$ = natural death rate
$q(\mathbf{r},t)$ = catchability
$f(\mathbf{r},t)$ = fishing death rate field = $q(\mathbf{r},t)\, e(\mathbf{r},t)$
$z(\mathbf{r},t) = m(\mathbf{r},t) + f(\mathbf{r},t)$
$N_T(\mathbf{r}_T,t_T)$ = number of tagged fish released at space-time point $(\mathbf{r}_T,t_T)$
$N_R$ = number of tagged fish recaptured in the interval of time $\mathcal{T}_T$
$\mathcal{L}_0$ = spatial differential operator dependent on $f$
$\mathcal{L}$ = spatial differential operator independent of $f$
$\mathcal{N}(\mathbf{r}',\sigma^2)$ = two-dimensional normal distribution centered at $\mathbf{r}' = x'\mathbf{i} + y'\mathbf{j}$ and variance $\sigma^2$
$\delta(\mathbf{r} - \mathbf{r}') = \delta(x - x')\delta(y - y')$ = two-dimensional Dirac delta function which can be represented as $\lim_{\sigma \to 0} \mathcal{N}(\mathbf{r}',\sigma^2)$
$g_0(\mathbf{r},t|\mathbf{r}',t')$ = effort-dependent Green function, which represents the probability density that a fish will survive the movement to point $\mathbf{r}$ from point $\mathbf{r}'$ in the interval of time $\mathcal{T}'$ when fishing takes place during that time interval
$g(\mathbf{r},t|\mathbf{r}',t')$ = effort-independent Green function, which represents the probability density that a fish will survive the movement to point $\mathbf{r}$ from point $\mathbf{r}'$ in the interval of time $\mathcal{T}'$ if no fishing takes place during that time interval
$r(\mathbf{r},t)$ = recruitment density rate field
$u_0(\mathbf{r},t)$ = population density due to recruitment = superposed recruitment density rate field with the effort-dependent Green function $g_0$
$u(\mathbf{r},t)$ = population density due to recruitment = superposed recruitment density rate field with the effort-independent Green function $g$
$h(t - t')$ = Heaviside step function which can be represented as $dh(t - t')/dt = \delta(t - t')$
$\mathbf{v}(\mathbf{r},t)$, $\mathbf{v}(t) = v_x\mathbf{i} + v_y\mathbf{j}$ = drift velocity
$k(\mathbf{r},t)$, $k(t)$ = diffusivity
$\mathcal{E} = \{e(\mathbf{r}_i,t_j) : i = 1, 2, \ldots, n;\ j = 1, 2, \ldots, m + 1\}$ = set of effort density measurements
$\mathcal{C} = \{c(\mathbf{r}_i,t_j) : i = 1, 2, \ldots, n; j = 1, 2, \ldots, m + 1\}$ = set of catch density rate measurements of untagged fish
$\mathcal{C}_T(\mathbf{r}_k,t_l) = \{c_T(\mathbf{r}_i,t_j) : i = 1, 2, \ldots, n; j = l, l + 1, \ldots, m + 1\}$ = set of catch density rate measurements of tagged fish released in cell $\mathcal{A}_k$ in the interval of time $\mathcal{T}_l$.
$\mathcal{C}_T = \{\mathcal{C}_T(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$ = set of catch density rate measurement of tagged fish for $n$ tagged fish releases in zones $\mathcal{A}_s$, correspondingly in the intervals of time $\mathcal{T}_{l+s-1}$ for $s = 1, 2, \ldots, n$.
$\mathcal{Q} = \{q(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$ = set of catchabilities computed from tagging experiments in cells $\mathcal{A}_s$ correspondingly in the intervals of time $\mathcal{T}_{l+s-1}$ for $s = 1, 2, \ldots, n$
$\mathcal{R} = \{r(\mathbf{r}_i,t_j) : i = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m + 1\}$ =

set of recruitment density rates computed at $\mathcal{A}_i$ in the intervals of time $\mathcal{T}_j$ for $j = l + n, l + n + 1, \ldots, m$

$\mathcal{G}_0(\mathbf{r}_k,t_l) = \{g_0(\mathbf{r}_i,t_{l+s}|\mathbf{r}_k,t_l) : i = 1, 2, \ldots, n; s = 1, 2, \ldots, m - l + 1\}$ = set of values of the effort-dependent Green function computed from a release of tagged fish in cell $\mathcal{A}_k$ in the interval of time $\mathcal{T}_l$

$\mathcal{G}_0 = \{\mathcal{G}_0(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$ = set of values of $n$ Green functions from tagged fish releases in cells $\mathcal{A}_s$ correspondingly during the intervals of time $\mathcal{T}_s$ for $s = l, l + 1, \ldots, l + n - 1$

$\mathcal{G}_0^*(\mathcal{T}_j) = \{g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j) : i, k = 1, 2, \ldots, n\}$ = set of effort-dependent transition probability densities for the interval of time $\mathcal{T}_j$

$\mathcal{G}_0^* = \mathcal{G}_0^*(\mathcal{T}_{l+n}) \cup \mathcal{G}_0^*(\mathcal{T}_{l+n+1}) \cup \ldots \cup \mathcal{G}_0^*(\mathcal{T}_m) = \{g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j) : i, k = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$ = set of effort-dependent transition probability densities in the intervals of time $\mathcal{T}_j$ for $j = l + n, l + n + 1, \ldots, m$

$\mathcal{G}^* = \{g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j) : i, k = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$ = set of effort-independent transition probability densities in the intervals of time $\mathcal{T}_j$ for $j = l + n, l + n + 1, \ldots, m$

$$\langle f(\mathbf{r}) \rangle\ (t|\mathbf{r}',t') = \int_{\mathcal{A}} d^2\mathbf{r}\, f(\mathbf{r}) g(\mathbf{r},t|\mathbf{r}',t')$$

$$\langle f(\mathbf{r}_i) \rangle\ (t_{j+1}|\mathbf{r}_k,t_j) = \sum_{i=1}^{n} \mathcal{A}_i f(\mathbf{r}_i) g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j)$$

l.h.s., r.h.s. = left-hand side, right-hand side.

## 2. Problem Statement and Formulation

Assume that almost all of a fish population is contained in a domain $\mathcal{A} \in \mathbf{R}^2$. Although in reality, fish populations inhabit three-dimensional space, this work will deal with highly migratory species whose vertical movement is negligible compared with their horizontal motion. Such is the case, for example, of pelagic species of fish.

Assume also that the population is being exploited with effort density $e(\mathbf{r},t)$ over the time interval $\mathcal{T}_I = [t_I,t] = t - t_I$. During that time interval, a catch density rate $c(\mathbf{r},t)$ is realized. Because I wish to determine how an increased fishing effort in a portion of $\mathcal{A}$ will affect the catch density rate in another portion of $\mathcal{A}$, the movement patterns of the population must be determined. To this end, a tagging experiment with distinguishable tags is initiated at time $t_T \in \mathcal{T}_I$ which results in a tagged fish catch density rate $c_T(\mathbf{r},t)$ in the interval of time $\mathcal{T}_T = [t_T,t] \subseteq \mathcal{T}_I$. In addition, I wish to calculate the recruitment density rate $r(\mathbf{r},t)$ and estimate parameters assuming that the fish population is accurately modeled by a diffusive population that drifts.

I have previously considered the fisheries interaction problem formulated above making no use of differential equations by applying the principle of linear superposition (Salvadó 1994). Here, I will go about solving this problem by use of the Green functions associated with population field equations derived from the Rayleigh transport theorem (Okubo 1980). This theorem states that any population that is in motion and distributed in a space–time continuum must satisfy the relationship

$$(1)\quad \frac{\partial p(\mathbf{r},t)}{\partial t} + \nabla\cdot\mathbf{j}(\mathbf{r},t) = s(\mathbf{r},t)$$

where $p$ is the population density, $\mathbf{j}$ is the flux of $p$ (the amount of transport of population across a unit area in unit time), and $s$ is the source (+) or sink (−) rate densities of $p$ at space–time point $(\mathbf{r},t)$. The flux $\mathbf{j}$ will be assumed to be the result of an operation on the spatial distribution of $p$ that results in a differential expression that is linear in $p$.

For a population whose movement and distribution in space–time are structured by age, the transport equation given in (1) must be modified. If $p(\mathbf{r},t,a)$ is the population density of individuals of age $a$ at space–time point $(\mathbf{r},t)$, the transport equation is given by (Murray 1989)

$$(2)\quad \frac{\partial p(\mathbf{r},t,a)}{\partial t} + \frac{\partial p(\mathbf{r},t,a)}{\partial a} + \nabla\cdot\mathbf{j}(\mathbf{r},t,a) = s(\mathbf{r},t,a)\,.$$

However, because aging is at the same rate as the passing of time, transforming (2) with the set of transformations for $t > a$

$$t' = t$$
$$a' = t - a$$
$$\mathbf{r}' = \mathbf{r}$$

leads to the transport equation

$$(3)\quad \frac{\partial p(\mathbf{r}',t',t'-a')}{\partial t'} + \nabla'\cdot\mathbf{j}(\mathbf{r}',t',t'-a') = s(\mathbf{r}',t',t'-a')\,.$$

Equation (3) is of the same form as (1) except that the latter has fields with two arguments of time. It follows that the solutions of the age-dependent field equations can be inferred from those expressions corresponding to age-independence. Therefore, for simplicity, this exposition will be for the age-independent case.

A population density is said to be conserved if the source and/or sink term $s$ in Eq. (1) vanishes, but in the general treatment here, population density may be nonconservative. With $d(\mathbf{r},t)$ as the natural death density rate, and $r(\mathbf{r},t)$ as the recruitment density rate, $s(\mathbf{r},t)$ is generally given by

$$s(\mathbf{r},t) = r(\mathbf{r},t) - [d(\mathbf{r},t) + c(\mathbf{r},t)]\,.$$

The biomass volume of fish populations is small in comparison with the volume of water they inhabit and, therefore, I shall assume that the population is dilute. As a consequence of this assumption, I can make some linear approximations. Because the catch density rate must be a function of population density (i.e., there can be no catch if there are no fish), expanding $c(p)$ in a Taylor series about $p = 0$ up to the linear term yields $c(p) = fp$ ($c(0) = 0$ necessarily), where $f = [\partial c(p)/\partial p]_{p=0}$ is the death rate due to fishing. Because $f$ must be a function of effort density (i.e., there can be no fishing death if there is no fishing), expanding $f(e)$ in a Taylor series about $e = 0$ up to the linear term yields $f(e) = qe$ ($f(0) = 0$ necessarily), where $q = [\partial^2 c(p,e)/\partial p \partial e]_{p=e=0}$ is the catchability. Therefore, for low population and effort densities, one can assume the model for catch density rate to be

$$(4)\quad c(\mathbf{r},t) = f(\mathbf{r},t)\,p(\mathbf{r},t) = q(\mathbf{r},t)\,e(\mathbf{r},t)\,p(\mathbf{r},t)\,.$$

Similarly, the natural death density rate $d$ must be a function of population density. Therefore, with $m$ the natural death rate (i.e., natural mortality) and $d(p)$ expanded in a Taylor series about $p = 0$ up to the linear term (where $d(0) = 0$ necessarily), at low population density the model for natural death density rate can

be assumed to be

$$(5)\quad d(\mathbf{r},t) = m(\mathbf{r},t)\,p(\mathbf{r},t)\,.$$

In the method of analyzing the dynamics of populations in space–time that follows, it is not necessary to invoke a particular model for the source term corresponding to recruitment density rate. However, as I shall show, it is possible to determine empirically the recruitment density rate once the population density due to recruitment is calculated.

I shall further assume that the population drifts with velocity $\mathbf{v}(\mathbf{r},t)$. The population is also assumed to disperse diffusively with diffusivity $k(\mathbf{r},t)$. Then, consistent with the assumption of linearity due to diluteness used in the formulation of the catch density rate and natural death density rate, the flux of the population density is

$$(6)\quad \mathbf{j}(\mathbf{r},t) = \left[\mathbf{v}(\mathbf{r},t) - k(\mathbf{r},t)\nabla\right] p(\mathbf{r},t)\,.$$

Therefore, the field equation the population density is assumed to satisfy is

$$(7)\quad \left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] p(\mathbf{r},t) = r(\mathbf{r},t)$$

in the spatial domain $\mathbf{r} \in \mathcal{A}$, where $\mathcal{L}_0$ is a differential operator that operates only on the spatial distribution of $p(\mathbf{r},t)$ and is given by

$$(8)\quad \mathcal{L}_0 = b_0(\mathbf{r},t) + \mathbf{b}_1(\mathbf{r},t)\cdot\nabla + b_2(\mathbf{r},t)\,\triangle$$

where

$$\nabla = \mathbf{i}\frac{\partial}{\partial x} + \mathbf{j}\frac{\partial}{\partial y}$$

$$\triangle = \nabla\cdot\nabla = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}$$

$$(9)\quad b_0(\mathbf{r},t) = z(\mathbf{r},t) + \nabla\cdot\mathbf{v}(\mathbf{r},t)$$

$$b_1(\mathbf{r},t) = \mathbf{v}(\mathbf{r},t) - \nabla\, k(\mathbf{r},t)$$

$$b_2(\mathbf{r},t) = -k(\mathbf{r},t)$$

and $z(\mathbf{r},t) = m(\mathbf{r},t) + f(\mathbf{r},t)$. The restrictions on the parameters of the field equation are that $\int_{t_T}^{t} d\tau z(\mathbf{r},\tau) \geq 0$, $\int_{t_T}^{t} d\tau k(\mathbf{r},\tau) \geq 0$, and that $\nabla\cdot\mathbf{v}(\mathbf{r},t)$ and $\triangle k(\mathbf{r},t)$ exist. For $b_0(\mathbf{r},t) = r(\mathbf{r},t) = 0$, field equation (7) is a Fokker–Planck equation used in the study of stochastic processes where probability is conserved (Gardiner 1990). In the general treatment of this work, $b_0(\mathbf{r},t) \neq 0$ and $r(\mathbf{r},t) \neq 0$. Because these terms represent sources and/or sinks of population, probability will not be conserved.

The domain of the population is finite, so I must discuss the boundary $\partial\mathcal{A}$ of $\mathcal{A}$. For the purposes of this paper the boundary $\partial\mathcal{A}$ is defined as the line at the edge of $\mathcal{A}$ (i.e., $\partial\mathcal{A} \subset \mathcal{A}$) beyond which the population is unlikely to inhabit due to unfavorable physicobiological conditions. In general, fish populations have two types of boundaries: well-defined ones delineating, for example, the water–land interface and fuzzy ones demarcating, for example, lines beyond which if a member of the population strays is likely to perish due to unfavorable environmental conditions. It can be shown (Appendix A) that with a prescribed population density at an initial time (initial condition) the solution of (7) is unique and "simply" represented when $p(\mathbf{r},t)$ or the component normal to $\partial\mathcal{A}$ of $\nabla p(\mathbf{r},t)$ vanish on $\partial\mathcal{A}$. By "simple" representation of the solution, I mean that the Green function associated with (7) is a simple function of measurable quantities, a concept that will become evident when I discuss the solutions. However, the required simplicity does lead to physicobiological pertinent boundaries of fish population domains.

Consider the first boundary condition

$$(10)\quad p(\mathbf{r},t) = 0$$

for $\mathbf{r} \in \partial\mathcal{A}$. The drift $\mathbf{v}(\mathbf{r},t)$ could be the motion of the medium, relative to a fixed coordinate system, that the population is riding or fighting. The diffusivity $k(\mathbf{r},t)$ is ultimately an environmental variable that could be negatively correlated with the amount of food encountered by the population at space–time point $(\mathbf{r},t)$. Low food availability in a region of the population domain would mean that the random motion of a foraging fish population in that region could increase and, according to (7), would be "pushed" in the opposite direction in which $k(\mathbf{r},t)$ changes the fastest towards a region of lower $k$. As I shall later show in an example, an increased diffusivity at a particular space–time point results in a decrease of the population density at that point. Although the natural death rate $m(\mathbf{r},t)$ has limits imposed by evolution, it is dependent also on environmental factors such as food availability and probability of encounters with predators. A high value for $m(\mathbf{r},t)$ could mean low food availability and/or high probability of encounter with a predator at space–time point $(\mathbf{r},t)$. Therefore, the boundary condition (10) would be a good model of a "fuzzy" population boundary preceded by regions of increasing diffusivity and/or natural death rate. A boundary on which (10) holds is aptly known as the perfectly absorbing boundary (Okubo 1980; Gardiner 1990). In the mathematical literature, this boundary condition is known as homogeneous Dirichlet conditions.

A second boundary condition mentioned above that leads to unique simple solutions of (7) (together with an initial condition) is formulated by considering the integral over $\mathcal{A}$ of equation (1) and applying the Divergence Theorem (Courant and Hilbert 1953) to the term containing the divergence of $\mathbf{j}(\mathbf{r},t)$. Letting $\mathbf{n}_\perp$ be the unit vector normal to $\partial\mathcal{A}$ pointing outward, then

$$\int_{\mathcal{A}} d^2\mathbf{r}\frac{\partial p(\mathbf{r},t)}{\partial t} = \int_{\mathcal{A}} d^2\mathbf{r}s(\mathbf{r},t) - \oint_{\partial\mathcal{A}} dl\,\mathbf{n}_\perp\cdot\mathbf{j}(\mathbf{r},t)\,.$$

This states that the change in population density over time is due to the sinks and sources of population in $\mathcal{A}$ and the flux of $p(\mathbf{r},t)$ across $\partial\mathcal{A}$. However, because $\mathcal{A}$ is the domain of the population, the flux of $p$ across $\partial\mathcal{A}$ must vanish and the change in population density is due to the sources and sinks. Therefore,

$$\mathbf{n}_\perp\cdot\mathbf{j}(\mathbf{r},t) = 0$$

for $\mathbf{r} \in \partial\mathcal{A}$. Using (6), it can be written as

$$\mathbf{n}_\perp \cdot \nabla p(\mathbf{r},t) = \frac{\mathbf{n}_\perp \cdot \mathbf{v}(\mathbf{r},t)}{k(\mathbf{r},t)} p(\mathbf{r},t)$$

in the domain $\mathbf{r} \in \partial\mathcal{A}$. Furthermore, because $\mathcal{A}$ is the domain of the population the vanishing of the flux must be due to the inability of any member of the population to cross $\partial\mathcal{A}$. Therefore, the component of the drift velocity normal to $\partial\mathcal{A}$ must also vanish (i.e., $\mathbf{n}_\perp \cdot \mathbf{v}(\mathbf{r},t) = 0$ for $\mathbf{r} \in \partial\mathcal{A}$) and the boundary condition reduces to

$$(11) \quad \mathbf{n}_\perp \cdot \nabla p(\mathbf{r},t) = 0$$

for $\mathbf{r} \in \partial\mathcal{A}$. A boundary on which (11) holds is aptly known as the perfectly reflecting boundary (Okubo 1980; Gardiner 1990). In the mathematical literature, this boundary condition is known as homogeneous Neumann conditions. This boundary condition is appropriate for the ocean–land boundary.

I shall assume throughout this work that the boundary of the domain of the population is composed of segments or portions which are perfectly absorbing and the remainder perfectly reflecting. If $\partial\mathcal{A}_A$ is the total of the absorbing segments, and $\partial\mathcal{A}_R$ is the total of the reflecting segments, then $\partial\mathcal{A} = \partial\mathcal{A}_A + \partial\mathcal{A}_R$. The solution of the field equation (7), with a prescribed initial condition, satisfying the mixed boundary conditions is unique (Appendix A).

Using this formulation, I shall show that data from a tagging experiment properly performed may be used to construct an approximate representation of a Green function. I shall, therefore, develop an empirical technique to construct Green functions using tagged fish catch and effort data and use these results for a fishery interaction problem and the calculation of model parameters.

## 3. Problem Solution

In this section, I will develop the Green function as an approximate representation of a tagging experiment, list its properties, and use it to represent the tagged and untagged fish population densities. For an intuitive approach to the developments in this section which makes no use of differential equations, see Salvadó (1994).

### 3.1. Effort-Dependent Green Function

In this subsection, I formulate and find representations of the solutions for the tagged and untagged portion of the population in terms of a point-source solution that is dependent on the death rate due to fishing. I shall also show how the point-source solutions are constructed from the fields $c_T$ and $e$. Although these are correct solutions to model the population field at one level of fishing effort, unless $m(\mathbf{r},t) \gg f(\mathbf{r},t)$, they are not of much use for the case of considering the interaction between different cells (for the time being defined as a small subset of $\mathcal{A}$) of the domain of the population at various levels of effort.

#### *3.1.1. Tagged fish dynamics*

Consider a tagging experiment in which $N_T$ tagged fish are released in an area small compared with $\mathcal{A}$ about position $\mathbf{r}_T$ beginning at time $t_T \in \mathcal{T}_T$. The duration of the tagged fish release is short conpared with $\mathcal{T}_T$, where $t \gg t_T$. I shall index the number of tagged fish, location, and initial time of release by writing $N_T(\mathbf{r}_T,t_T)$.

Let $\mathcal{L}_0$ be a linear differential operator which contains the terms of the field equation that describe the natural and fishing death rates and the spatial dynamics. Consistent with (7) and the general fish population problem formulated in Section 2, the tagged fish population density $p_T(\mathbf{r},t)$ must satisfy a field equation of the form

$$(12) \quad \left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] p_T(\mathbf{r},t) = 0$$

in the space–time domain $\mathbf{r} \in \mathcal{A}$, $t \geq t_T$.

The initial condition of the tagged fish release process will be approximated as if the release is at one point (i.e., at $\mathbf{r} = \mathbf{r}_T$) and instantaneous (i.e., at $t = t_T$): an approximate representation of the initial condition of the population density of tagged fish is

$$(13) \quad p_T(\mathbf{r},t_T) = N_T(\mathbf{r},t_T)\,\delta(\mathbf{r} - \mathbf{r}_T)$$

in the space domain $\mathbf{r} \in \mathcal{A}$ and where $\delta$ is a two-dimensional Dirac delta function (Lighthill 1964; Butkov 1968). The discretization of space–time will relax the condition of an instantaneous release at a point of all the tagged fish.

The boundary $\partial\mathcal{A} = \partial\mathcal{A}_A + \partial\mathcal{A}_R$ is assumed composed of segments on which it is absorbing ($\partial\mathcal{A}_A$) and those on which it is reflecting ($\partial\mathcal{A}_R$). Therefore, $p_T(\mathbf{r},t)$ satisfies

$$(14) \quad p_T(\mathbf{r},t) = 0$$

on the space–time domain $\mathbf{r} \in \partial\mathcal{A}_A$, $t \geq t_T$, and

$$(15) \quad \mathbf{n}_\perp \cdot \nabla p_T(\mathbf{r},t) = 0$$

in the space–time domain $\mathbf{r} \in \partial\mathcal{A}_R$, $t \geq t_T$.

I seek the solution of (12) that satisfies conditions (13), (14), and (15). To this end, I consider the Green function that is associated with (12) for a source space–time point at $(\mathbf{r}',t')$. With respect to the receiver space–time coordinates $(\mathbf{r},t)$, the Green function associated with (12) satisfies (Appendix A)

$$(16) \quad \left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] g_0(\mathbf{r},t|\mathbf{r}',t') = \delta(\mathbf{r}-\mathbf{r}')\,\delta(t-t')$$

in the domain $\mathbf{r} \in \mathcal{A}$, a causality condition

$$(17) \quad g_0(\mathbf{r},t|\mathbf{r}',t') = 0 \text{ if } t < t'$$

in the domain $\mathbf{r} \in \mathcal{A}$, and boundary conditions

$$g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_A$ and

$$\mathbf{n}_\perp \cdot \nabla g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_R$.

The solution that satisfies (12), (13), (14), and (15) is given by (Appendix A)

$$(18) \quad p_T(\mathbf{r},t) = g_0(\mathbf{r},t|\mathbf{r}_T,t_T)\,N_T(\mathbf{r}_T,t_T)\,.$$

As an example, if $\mathcal{L}_0$ is given by

$$\mathcal{L}_0 = z(t) + \mathbf{v}(t) \cdot \nabla - k(t)\,\Delta\,, \tag{19}$$

then the solution of (16) in an unbounded domain that satisfies the causality condition (17) and boundary condition

$$\lim_{r \to \infty} g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

where $r = \sqrt{\mathbf{r} \cdot \mathbf{r}}$ for a source at $(\mathbf{r}_T,t_T)$ is

$$g_0(\mathbf{r},t|\mathbf{r}_T,t_T) = \exp\left[-\int_{\mathcal{T}_T} d\tau z(\tau)\right] \times \mathcal{N}\left(\mathbf{r}_T + \int_{\mathcal{T}_T} d\tau \mathbf{v}(\tau), \sigma^2\right) h(t - t_T)\,, \tag{20}$$

$\mathcal{N}(\mathbf{r}_T,\sigma^2)$ is the normal distribution centered at $\mathbf{r}_T$ with variance $\sigma^2$ given by

$$\sigma^2(t,t_T) = 2\int_{\mathcal{T}_T} d\tau k(\tau)\,, \tag{21}$$

and $h(t - t_T)$ is the Heaviside step function defined as

$$h(t - t_T) = \begin{cases} 0 \text{ if } t < t_T \\ 1 \text{ if } t \geq t_T\,. \end{cases}$$

As can be appreciated the amplitude of (20) decreases when $m$ and/or $k$ increase. It is initially (i.e., at $t = t_T$) a normal distribution centered at $\mathbf{r} = \mathbf{r}_T$ with vanishing variance (i.e., a Dirac delta function). For $t < t_T$ the distribution vanishes, while for $t > t_T$ the distribution spreads at a rate that depends on the diffusion coefficient $k$, and the mean drifts at a rate determined by the drift velocity $\mathbf{v}$. While the distribution $\mathcal{N}$ is normalized for all $t \geq t_T$, the distribution $g_0$ does not conserve probability. Because $z(t) = m(t) + f(t) > 0$, the exponential function or survivability coefficient (Ricker 1975) leading $\mathcal{N}$ in (20) decreases with increasing time from an initial value of unity. I shall show that these properties of $g_0$ are general properties of the point-source solutions associated with (19). To see this, I solve for $g_0$ from (18). With the use of (4), I can express $g_0$ as a function of the measured fields $e$ and $c_T$ as

$$g_0(\mathbf{r},t|\mathbf{r}_T,t_T) = \frac{p_T(\mathbf{r},t)}{N_T(\mathbf{r}_T,t_T)} = \frac{c_T(\mathbf{r},t)}{q(\mathbf{r},t)\,e(\mathbf{r},t)\,N_T(\mathbf{r}_T,t_T)}\,. \tag{22}$$

Because I know the number $N_T$ of tagged fish released at space–time point $(\mathbf{r}_T,t_T)$, and I have $c_T(\mathbf{r},t)$ and $e(\mathbf{r},t)$ in the interval of time $\mathcal{T}_T$, I can construct empirically the Green function $g_0(\mathbf{r},t|\mathbf{r}_T,t_T)$ in the interval of time $\mathcal{T}_T$ if I have $q(\mathbf{r},t)$.

The Green function $g_0$ has a series of properties that can be inferred. The first of these properties will allow the estimation of $q(\mathbf{r},t)$ as follows.

*A. Initial* — Assuming tagged fish disperse at a finite rate, initially they must be present at the point of release only. Therefore, for a source space-time point at $(\mathbf{r}_T,t_T)$, $g_0$ must satisfy

$$g_0(\mathbf{r},t_T|\mathbf{r}_T,t_T) = \delta(\mathbf{r} - \mathbf{r}_T)\,.$$

Analytically this can be deduced by dividing through (13) by the number of tagged fish released. Therefore, multiplying expression (22) by $q(\mathbf{r},t)$ and integrating over the spatial domain and application of the initial property of $g_0$ for $t \to t_T$ yields

$$q(\mathbf{r}_T,t_T) = \frac{1}{N_T(\mathbf{r}_T,t_T)} \int_{\mathcal{A}} d^2\mathbf{r}\, \frac{c_T(\mathbf{r},t_T)}{e(\mathbf{r},t_T)}\,. \tag{23}$$

Dimensional analysis of (23) reveals that $q$ has the dimensions of area × time$^{-1}$. The dependence of its value on spatiotemporal location will ultimately dictate in how many cells and intervals of time must tagged fish be released in order to characterize the pattern of fish movement within $\mathcal{A}$. The empirical construction of $g_0(\mathbf{r},t_T|r',t')$ requires knowledge of $q(\mathbf{r},t)$ for all $\mathbf{r} \in \mathcal{A}$ and all $t \in \mathcal{T}_T$. The calculation of $q$ by (23) requires that it be done at the space–time point of the tagged fish release. It follows, therefore, that to construct $q(\mathbf{r},t)$ for all $\mathbf{r} \in \mathcal{A}$ and all $t \in \mathcal{T}_T$ in principle requires performing nondenumerably infinite tagging experiments if $q$ depends strongly on $(\mathbf{r},t)$. However, as will be discussed later, discretization and weak dependence of $q$ on $(\mathbf{r},t)$ will allow some approximations.

The accurate estimation of $q$ using (23) requires that the loss of tagged fish due to natural mortality during the time of release be negligible. It is, therefore, important to release the tagged fish in as short a time as possible. If $[t_T, t_{T+\delta}]$ is the interval of time over which the tagged fish are released, the required condition of small loss of tagged fish due to natural death rate during the time of release will be fullfilled if $m(\mathbf{r},t_T) \ll 1/[t_T,t_{T+\delta}]$. Other properties of $g_0$ are as follows.

*B. Dimensional* — Dimensional analysis of (22) reveals that $g_0$ is a density. That is, $g_0$ has the dimensions of area$^{-1}$.

*C. Causal* — Because tagged fish can exist in their domain only if they are present at an equal or earlier time, for a source time $t_T$, $g_0$ must satisfy

$$g_0(\mathbf{r},t|\mathbf{r}_T,t_T) = 0 \text{ for } t < t_T\,.$$

*D. Normalization* — As a consequence of the initial property,

$$\int_{\mathcal{A}} d^2\mathbf{r}\, g_0(\mathbf{r},t_T|\mathbf{r}_T,t_T) = 1\,.$$

*E. Probabilistic* — Because the number of tagged fish recaptured cannot be greater than those released, for $t \geq t_T$,

$$0 \leq g_0(\mathbf{r},t|\mathbf{r}_T,t_T) \leq \delta(\mathbf{r} - \mathbf{r}_T)$$

and therefore,

$$0 \leq \int_{\mathcal{A}} d^2\mathbf{r}\, g_0(\mathbf{r},t|\mathbf{r}_T,t_T) \leq 1\,.$$

Hence, $g_0(\mathbf{r},t|\mathbf{r}_T,t_T)$ can be regarded as the probability density of a tagged fish surviving the movement to position $\mathbf{r}$ from $\mathbf{r}_T$ during the interval of time $\mathcal{T}_T$ when fishing takes place in that interval of time. Assuming that the recaptured tagged fish are not rereleased, I will call $g_0$ the effort-dependent Green function.

*F. Nonconservation of probability* — Because tagged fish are being removed from their domain by natural and fishing deaths,

in general it must be true that for $t > t_T$,

$$\int_{\mathcal{A}} d^2\mathbf{r}\, g_0(\mathbf{r},t|\mathbf{r}_T,t_T) < 1 .$$

In order to derive the property of linear superposition of Green functions, consider the solution (18) which I will write as

$$(24)\quad p_T(\mathbf{r}',t') = g_0(\mathbf{r}',t'|\mathbf{r}_T,t_T)\, N_T(\mathbf{r}_T,t_T) .$$

Suppose I wanted to use the final condition $p_T(\mathbf{r}',t')$ of (24) as the initial condition of a tagged population problem. The population density must then satisfy field equation (12) and initial condition

$$p_T(\mathbf{r}',t') = \frac{c_T(\mathbf{r}',t')}{q(\mathbf{r}',t')\, e(\mathbf{r}',t')}$$

for $\mathbf{r}' \in \mathcal{A}$ (or some other estimate at $(\mathbf{r}',t')$ of $p_T$) and boundary conditions (14) and (15). The solution is given by (Appendix A)

$$(25)\quad p_T(\mathbf{r},t) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, p_T(\mathbf{r}',t') .$$

Dividing (25) by $N_T(\mathbf{r}_T,t_T)$ and using the definition of $g_0$ given by (22) leads to the following property.

*G. Linear superposition of probability densities* — The probability density satisfies

$$(26)\quad g_0(\mathbf{r},t|\mathbf{r}_T,t_T) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, g_0(\mathbf{r}',t'|\mathbf{r}_T,t_T) .$$

Another important property of $g_0$ is its periodicity.

*H. Periodicity* — A Green function is said to be periodic in time, of period $\mathcal{T}$, if $\mathcal{T}$ is a constant such that

$$g(\mathbf{r},t|\mathbf{r}',t') = g(\mathbf{r},t+\mathcal{T}|\mathbf{r}',t'+\mathcal{T})$$

(Tolstov 1962). A spatial periodicity can also be defined.

*3.1.2. Untagged fish dynamics*

Consistent with (7) and the general fishery problem formulated in the foregoing section, I would like to find expressions for the population density of the untagged portion in the interval of time $\mathcal{T}_I$. However, because the empirical Green function is constructed for $t \geq t_T \geq t_I$, in general it is not possible to define empirically the population density in the interval of time $t_I \leq t \leq t_T$ from a tagging campaign initiated at time $t_T$. Therefore, I shall pose the problem for the untagged portion of the population in the interval of time $\mathcal{T}_T$.

The population density of untagged fish $p(\mathbf{r},t)$ obeys the field equation

$$(27)\quad \left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] p(\mathbf{r},t) = r(\mathbf{r},t)$$

in the space–time domain $\mathbf{r} \in \mathcal{A}$, $t \geq t_T$ subject to initial condition

$$(28)\quad p(\mathbf{r},t_T) = p_{in}(\mathbf{r}) = \frac{c(\mathbf{r},t_T)}{q(\mathbf{r},t_T)\, e(\mathbf{r},t_T)}$$

in the domain $\mathbf{r} \in \mathcal{A}$ (or some other estimate at $(\mathbf{r},t_T)$ of $p$) and boundary conditions

$$(29)\quad p(\mathbf{r},t) = 0$$

in the domain $\mathbf{r} \in \partial\mathcal{A}_A$, $t \geq t_T$ and

$$(30)\quad \mathbf{n}_\perp \cdot \nabla p(\mathbf{r},t) = 0$$

in the domain $\mathbf{r} \in \partial\mathcal{A}_R$, $t \geq t_T$.

Because the differential operators for the tagged and untagged portions of the fish population are identical, even though their inhomogeneous term and initial conditions differ, this means that the movement and survivability of tagged and untagged fish are linked through the equality of their Green functions. In practice, this is true only if sufficient numbers of tagged fish are released so that $g_0$ constructed as indicated by (22) approximately represents the average movement and survivability of the total fish population.

The solution that satisfies (27), (28), (29), and (30) is (Appendix A)

$$(31)\quad p(\mathbf{r},t) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + u_0(\mathbf{r},t)$$

where the field $u_0(\mathbf{r},t)$ is the contribution to the population density due to recruitment and is given by

$$(32)\quad u_0(\mathbf{r},t) = \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, r(\mathbf{r}',t') .$$

Multiplying (31) by the fishing death rate $f(\mathbf{r},t)$ yields the expression for the catch density rate:

$$(33)\quad c(\mathbf{r},t) = f(\mathbf{r},t)\left[\int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + u_0(\mathbf{r},t)\right]$$

The contribution to the population density due to recruitment at the level of exploitation at which the empirical probability density $g_0$ was constructed can be computed by use of (33). Solving for $u_0$, I have

$$(34)\quad u_0(\mathbf{r},t) = \frac{c(\mathbf{r},t)}{f(\mathbf{r},t)} - \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') .$$

Because in the interval of time $\mathcal{T}_T$, I know $f(\mathbf{r},t)$ and the resulting $c(\mathbf{r},t)$, (34) can be used to calculate $u_0(\mathbf{r},t)$ at the level of effort for which $g_0(\mathbf{r},t|\mathbf{r}',t_T)$ was constructed. However, in general, I cannot use equation (33) to compute the catch density rate at a new level of effort unless I perform another tagging experiment at the desired level. The survivability embedded in $g_0$ depends on the level of exploitation during the recapture of the tagged fish. Therefore, although these equations using $g_0$ are valid for modeling the movement and distribution of a fish population, they are in general useful only at the level of effort for which $g_0$ was constructed. In general, no other case can be rigourously considered. In Section 4, I shall show that if the natural death rate is much greater than that due to fishing, the equations derived in this section can be used for a fisheries interaction study. However, in order to be able to consider the general fisheries interaction problem formulated in Section 2 without having to perform tagging experiments for all the levels of effort that wish

to be considered, I need to reformulate the problem so that the solutions for the population density are in terms of a Green function that is effort independent.

### 3.2. Effort-Independent Green Function

In this subsection, I shall find the solutions for the population density for tagged and untagged fish in terms of a Green function that is effort independent. I shall also show how to construct the effort-independent Green function from knowledge of the effort-dependent one. This new Green function satisfies, other than the fact that it is independent of fishing death rate, properties identical to those of $g_0$.

#### *3.2.1. Tagged fish dynamics*

Consider again a tagging experiment in which $N_T$ tagged fish are released in an area small compared with $\mathcal{A}$ about position $\mathbf{r}_T$ beginning at time $t_T \in \mathcal{T}_I$ with a duration short compared with $\mathcal{T}_T$ where $t \gg t_T$. Here again, I shall index the number of tagged fish, location, and initial time of release by writing $N_T(\mathbf{r}_T,t_T)$. However, in this case, I shall pose the problem with a differential operator that is independent of the fishing death rate, and I shall pass on to the inhomogeneous part of the field equation the responsibility for embedding into the solution for the population density the information of catch density rate.

Let $\mathcal{L}$ be a linear differential operator which contains the terms of (7) that describe the natural death rate and the spatial dynamics. In terms of operator $\mathcal{L}_0$, operator $\mathcal{L}$ is given by

$$\mathcal{L} = \mathcal{L}_0 - f(\mathbf{r},t) .$$

Consistent with (7) and the general fishery problem formulated in Section 2, the tagged fish population density must therefore satisfy a field equation of the form

$$(35)\quad \left[\frac{\partial}{\partial t} + \mathcal{L}\right] p_T(\mathbf{r},t) = -c_T(\mathbf{r},t)$$

in the space–time domain $\mathbf{r} \in \mathcal{A}$, $t \geq t_T$, subject to initial condition (13) and boundary conditions (14) and (15). The tagged fish catch density rate is now the inhomogeneous term of the field equation. It is a negative contribution because it is a sink of population. The solution that satisfies (35), (13), (14), and (15) is given by (Appendix A)

$$(36)\quad p_T(\mathbf{r},t) = g(\mathbf{r},t|\mathbf{r}_T,t_T)\, N_T(\mathbf{r}_T,t_T) - \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t') .$$

I need to determine how the effort-independent Green function is related to $g_0$. On dividing equation (36) by $N_T$ and using the definition of $g_0$ given by (22), the relation between the effort-dependent and effort-independent Green functions becomes

$$(37)\quad g(\mathbf{r},t|\mathbf{r}_T,t_T) = g_0(\mathbf{r},t|\mathbf{r}_T,t_T) + \frac{1}{N_T(\mathbf{r}_T,t_T)} \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t')$$

which is an integral equation where $g(\mathbf{r},t|\mathbf{r}',t')$ is the unknown. The exact solution to integral equation (37) is given by the Neumann series (Courant and Hilbert 1953; Byron and Fuller 1970)

$$(38)\quad g(\mathbf{r},t|\mathbf{r}_T,t_T) = \sum_{i=0}^{\infty} g_i(\mathbf{r},t|\mathbf{r}_T,t_T)$$

where

$$(39)\quad g_i(\mathbf{r},t|\mathbf{r}_T,t_T) = \frac{1}{N_T(\mathbf{r}_T,t_T)} \int_{\mathcal{T}_T} dt' \times \int_{\mathcal{A}} d^2\mathbf{r}'\, g_{i-1}(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t')$$

for $i = 1, 2, \ldots$. The series (38) converges over an arbitrary interval of time whenever the number of tagged fish recaptured is less than the total number of tagged fish available at the beginning of the interval (Appendix B), and therefore, it converges uniformly to the solution under that condition (Byron and Fuller 1970).

To discuss some approximations, consider the total number of tagged fish recaptured in the interval of time $\mathcal{T}_T$:

$$N_R(t) = \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, c_T(\mathbf{r}',t') .$$

If $N_R < N_T$ but $N_R \not\ll N_T$, the approximation

$$g(\mathbf{r},t|\mathbf{r}_T,t_T) \approx g_0(\mathbf{r},t|\mathbf{r}_T,t_T) + g_1(\mathbf{r},t|\mathbf{r}_T,t_T) \approx g_0(\mathbf{r},t|\mathbf{r}_T,t_T) + \frac{1}{N_T(\mathbf{r}_T,t_T)} \int_{\mathcal{T}_T} dt' \times \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t')$$

can be used. However, if $N_R \ll N_T$, which implies $m(\mathbf{r},t) \gg f(\mathbf{r},t)$, then the approximation

$$g(\mathbf{r},t|\mathbf{r}_T,t_T) \approx g_0(\mathbf{r},t|\mathbf{r}_T,t_T)$$

is adequate. In a practical computational sense, the expansion is stopped at the term whose contribution is negligible.

The properties of $g$ can be determined with the help of (37). It satisfies all the properties of $g_0$ listed in Subsection (3.1.1.). However, because $g$ does not have fishing death rate included, the survivability associated with it must be in general of larger magnitude than that of $g_0$ except initially. It therefore satisfies

$$g(\mathbf{r},t|\mathbf{r}_T,t_T) \geq g_0(\mathbf{r},t|\mathbf{r}_T,t_T)$$

where the equality holds for $t = t_T$. Therefore, $g(\mathbf{r},t|\mathbf{r}_T,t_T)$ can be regarded as the probability density of a tagged fish surviving the movement to position $\mathbf{r}$ from $\mathbf{r}_T$ during the interval of time $\mathcal{T}_T$ in the absence of fishing during that interval of time. I will therefore call $g$ the effort-independent Green function.

On multiplying (36) by the fishing death rate, the tagged fish catch density rate is the solution of integral equation

$$c_T(\mathbf{r},t) = f(\mathbf{r},t)\left[g(\mathbf{r},t|\mathbf{r}_T,t_T)\, N_T(\mathbf{r}_T,t_T) - \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2r'\, g(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t')\right].$$

*3.2.2. Untagged fish dynamics*

Consistent with (7), the general fishery problem formulated in Section (2) and the fact that the empirical Green function is known only for $t \geq t_T$, the population density of untagged fish must obey a field equation

$$(40)\quad \left[\frac{\partial}{\partial t} + \mathcal{L}\right] p(\mathbf{r},t) = -c(\mathbf{r},t) + r(\mathbf{r},t)$$

in the space–time domain $\mathbf{r} \in \mathcal{A}$, $t \geq t_{T_0}$, and the conditions (28), (29), and (30). The solution is

$$(41)\quad p(\mathbf{r},t) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t_I)\, p_{in}(\mathbf{r}') + u(\mathbf{r},t) - \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, c(\mathbf{r}',t')$$

where

$$(42)\quad u(\mathbf{r},t) = \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, r(\mathbf{r}',t')$$

represents the contribution to the population density attributable to recruitment.

The untagged fish catch density rate is the solution of

$$(43)\quad c(\mathbf{r},t) = f(\mathbf{r},t)\left[\int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + u(\mathbf{r},t)\right] - f(\mathbf{r},t)\int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, c(\mathbf{r}',t')\,.$$

Because the catch density rate is known for the level of effort employed during the time interval $\mathcal{T}_T$, then (43) can be applied to those data to determine empirically the field $u$ using

$$(44)\quad u(\mathbf{r},t) = \frac{c(\mathbf{r},t)}{f(\mathbf{r},t)} - \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t')\, c(\mathbf{r}',t')\,.$$

Assuming that the recruitment does not change significantly at other levels of effort, catch density rate $c$ can then be computed for differing effort densities $e$ because (43) is valid for any level of effort as opposed to equation (33), which applies only to the measure of effort for which the effort-dependent Green function was constructed.

## 4. Example: Skipjack Tuna Fishery in the Eastern Tropical Atlantic

The following example shows how a subset of the type of fishery models considered in Section 3 can be reduced to a simple calculation whenever the effort density is sufficiently low. The fishery of interest is the skipjack tuna (*Katsuwonus pelamis*) fishery in the five cells of the eastern tropical Atlantic (ETA) indicated in Fig. 1. As a politically interesting question, A. Fonteneau (C.R.O.D.T., B.P. 2241, Dakar, Senegal) asks in a personal communication what effect the doubling of effort in one cell might have on the catches from the other four.

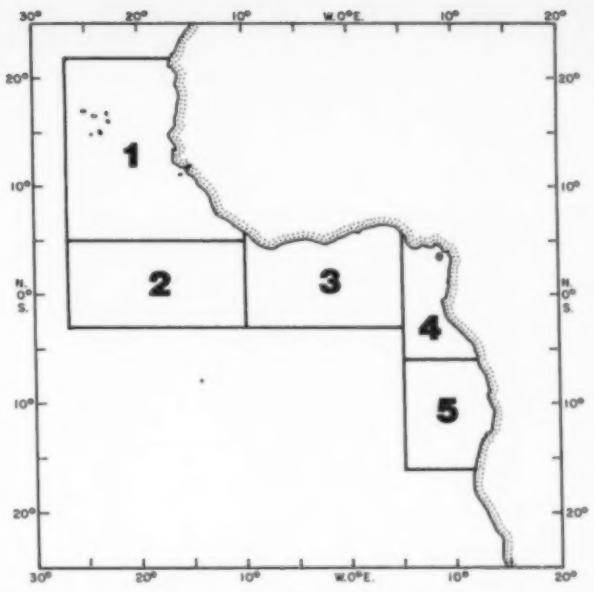



FIG. 1. Eastern tropical Atlantic (ETA) geographic cells: 1, Senegal; 2, Liberia; 3, Ghana; 4, Cape Lopez; 5, Angola.

The data required for the analysis consist solely of the mark and recapture measures from the International Skipjack Year Program, conducted over a 3-yr period by the International Commission for the Conservation of Atlantic Tunas (Symons et al. 1986). Although the corresponding catch and effort densities are also given in Symons et al. (1986), the only information needed here is the reported 5% tag return, together with the assumptions that almost all of the tagged fish survived the trauma of tagging and that almost all of the recaptured tags were reported. As I will show, the fishing effort expended during the experiment was sufficiently low to permit me to regard the dynamic catch density rate as approximately a linear function of effort density.

In the most extreme case, all of the $N_R = 5 \times 10^{-2} N_T$ recovered tags could have been taken in just one of the five cells over a single time interval corresponding to the space–time point $(\mathbf{r}_R, t_R)$ for $t_R \geq t_T$. Accordingly, the catch density rate of the tagged fish would then be $c_T(\mathbf{r},t) = N_R\delta(\mathbf{r} - \mathbf{r}_R)\delta(t - t_R)$. Therefore, on substituting those values in (37) and integrating, I can conclude that the difference between the effort-independent and effort-dependent Green functions is no greater than

$$g(\mathbf{r},t|\mathbf{r}_T,t_T) - g_0(\mathbf{r},t|\mathbf{r}_T,t_T) = 5 \times 10^{-2}\, g(\mathbf{r},t|\mathbf{r}_R,t_R)\,.$$

Because $5 \times 10^{-2} \ll 1$, $g \approx g_0$, which in turn implies that the natural death rate is much greater than the fishing death rate. In that limit, (33) becomes

$$c(\mathbf{r},t) \approx f(\mathbf{r},t)\left[\int_{\mathrm{ETA}} d^2\mathbf{r}'\, g(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + u(\mathbf{r},t)\right].$$

The catch density rate, under the condition of low fishing death rate in relation to the natural death rate, is approximately a linear function of effort density. Had the tag return been double its reported value, the approximation of catch as a linear function of effort would still hold because $N_R/N_T = 0.1$ is an order of

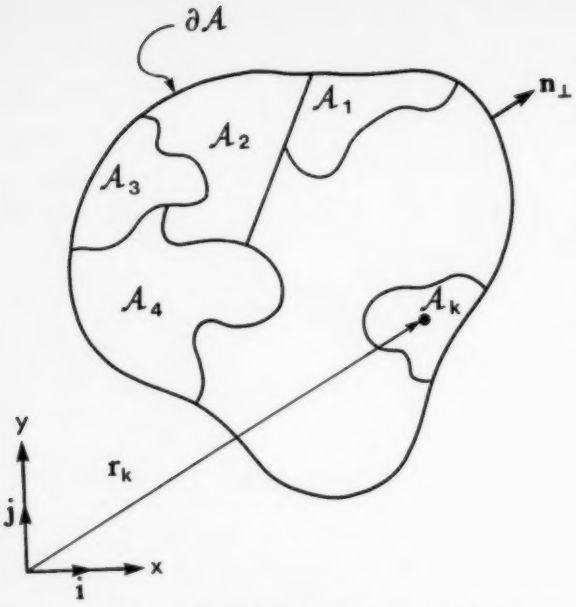



FIG. 2. Domain $\mathcal{A}$ of the population is divided into $n$ nonoverlapping cells. The vector $\mathbf{n}_\perp$ is the unit vector normal to the boundary $\partial\mathcal{A}$ of the domain pointing outward.

magnitude smaller than 1. Should the recruitment density rate not change sigificantly, doubling the effort would have the effect of approximately doubling the catch. Thus, in answer to the Fonteneau question, if the effort in one or more of the five cells were doubled, the catch from the remaining cells would remain approximately constant.

## 5. Discretization of the Fields

Should the fishing death rate approach or exceed the natural death rate, the field equations of Section 3 must be treated in much more detail to address the general fishery problem outlined in Section 2. A numerical solution is proposed in this section.

With reference to Fig. 2, the individual cells of area $\mathcal{A}_i$ of the domain $\mathcal{A}$, where $i = 1, 2, \ldots, n$, are such that

$$\mathcal{A} = \sum_{i=1}^{n} \mathcal{A}_i$$

(i.e., no overlap of cells), and $\{\mathbf{r}_i \in \mathcal{A}_i : i = 1, 2, \ldots, n\}$. Here, cell $\mathcal{A}_i$ is defined as a neighborhood around point (or nuclei) $\mathbf{r}_i$. A cell is defined about every data collection nucleus. An economic zone can be composed of more than one cell. The closed time interval $\mathcal{T}_T = [t_T, t] = [t_1, t_{m+1}]$, where $l \geq 1$ and the equality holds only if $\mathcal{T}_T = \mathcal{T}_l$. The $m$ time intervals $\{\mathcal{T}_j = t_{j+1} - t_j : j = 1, 2, \ldots, m\}$ are such that

$$\mathcal{T}_l = \sum_{j=1}^{m} \mathcal{T}_j = [t_1, t_{m+1}] .$$

The value of a field $\phi$ within cell $\mathcal{A}_i$ in the interval of time $\mathcal{T}_j = [t_j, t_{j+1}]$ will be written $\phi(\mathbf{r}_i, t_j)$. Henceforth, $(\mathbf{r}_i, t_j)$ is interchangeable with $(\mathcal{A}_i, \mathcal{T}_j)$.

I will assume that $N_T$ number of tagged fish are released in cell $\mathcal{A}_k$ at time $t_l$. The following discrete fields are available for the analysis: $\mathcal{E} = [e(\mathbf{r}_i, t_j) : i = 1, 2, \ldots, n; j = 1, 2, \ldots, m+1\}$, $\mathcal{C} = \{c(\mathbf{r}_i, t_j) : i = 1, 2, \ldots, n; j = 1, 2, \ldots, m+1\}$, and $\mathcal{C}_T(\mathbf{r}_k, t_l) = \{c_T(\mathbf{r}_i, t_j) : i = 1, 2, \ldots, n; j = l, l+1, \ldots, m+1\}$. These are, respectively, the measurements of effort density, the untagged fish catch density rate, and tagged fish catch density rate for the release of tagged fish in cell $\mathcal{A}_k$ at time $t_l$. It will soon become evident that at the very least there should be as many tagging experiments as there are cells in the domain, one per cell.

As I showed in Section 3, I first need to evaluate the catchability in order to construct the effort-dependent Green function. The discrete version of (23) is

$$(45)\quad q(\mathbf{r}_v, t_s) = \frac{1}{N_T(\mathbf{r}_v, t_s)} \sum_{i=1}^{n} \mathcal{A}_i \frac{c_T(\mathbf{r}_i, t_s)}{e(\mathbf{r}_i, t_s)} .$$

However, by its very definition, I can calculate only one value of $q$ from every tagging experiment. If the tagged fish release is only at $(\mathbf{r}_k, t_l)$, then I can only calculate $q(\mathbf{r}_k, t_l)$. So in the case of the single tagging experiment, I am left with the necessity of assuming $q$ constant throughout space–time.

The discrete version of (22) for $g_0$ for a tagged fish release at $(\mathbf{r}_k, t_l)$ is

$$(46)\quad g_0(\mathbf{r}_i, t_j | \mathbf{r}_k, t_l) = \frac{c_T(\mathbf{r}_i, t_j)}{q(\mathbf{r}_i, t_j)\, e(\mathbf{r}_i, t_j)\, N(\mathbf{r}_k, t_l)} .$$

If only the one tagging experiment has been performed, then, as mentioned above, it is necessary to assume $q(\mathbf{r}_i, t_j) = q(\mathbf{r}_k, t_l) =$ constant for $i = 1, 2, \ldots, n$ and $j = l, l+1, \ldots, m+1$. Thus, using the set $\mathcal{C}_T$ of a single tagging experiment and set $\mathcal{E}$, I can construct an approximate $g_0$. The set of values calculated for $g_0$ for a release of fish at $(\mathbf{r}_k, t_l)$ will be called the set $\mathcal{G}_0(\mathbf{r}_k, t_l) = \{g_0(\mathbf{r}_i, t_{l+s} | \mathbf{r}_k, t_l) : i = 1, 2, \ldots, n; s = 1, 2, \ldots, m-l+1\}$. To describe the elements of $\mathcal{G}_0(\mathbf{r}_k, t_l)$, it is helpful to use the Kroenecker delta function:

$$\delta_{ik} = \begin{cases} 1 & \text{if } i = k \\ 0 & \text{otherwise} . \end{cases}$$

Then

$$g_0(\mathbf{r}_i, t_j | \mathbf{r}_k, t_l) = \begin{cases} 0 & \text{for } t_j < t_l \\ \delta_{ik} / \mathcal{A}_k & \text{for } t_j = t_l \end{cases}$$

and

$$g_0(\mathbf{r}_i, t_j | \mathbf{r}_k, t_l) \leq \delta_{ik} / \mathcal{A}_k \quad \text{for } t_j > t_l .$$

As a consequence of this last property, for $t_j \geq t_l$:

$$0 \leq \sum_{i=1}^{n} \mathcal{A}_i\, g_0(\mathbf{r}_i, t_j | \mathbf{r}_i, t_l) \leq 1 .$$

The discrete version of (37) is given by

$$g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_l) \approx g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_l) + \frac{1}{N_T(\mathbf{r}_k,t_l)}$$

$$\times \sum_{s=1}^{fSm} \mathcal{T}_s \sum_{v=1}^{n} \mathcal{A}_v\, g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_v,t_s)\, c_T(\mathbf{r}_v,t_s)\,.$$

Therefore, for an arbitrary single interval of time, say $\mathcal{T}_j$, the relationship between $g$ and $g_0$ is

$$(47)\quad g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) \approx g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) + \frac{\mathcal{T}_j}{N_T(\mathbf{r}_w,t_j|\mathbf{r}_k,t_l)}$$

$$\times \sum_{v=1}^{n} \mathcal{A}_v\, g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_v,t_j)\, c_T(\mathbf{r}_v,t_j)$$

where $N(\mathbf{r}_w,t_j|\mathbf{r}_k, \mathbf{t}_l)$ is the number of tagged fish at $(\mathbf{r}_w,t_j)$ from a release conducted at $(\mathbf{r}_k,t_l)$. From (18), this is

$$N_T(\mathbf{r}_w,t_j|\mathbf{r}_k,t_l) = \mathcal{A}_w\, p_T(\mathbf{r}_w,t_j) = \mathcal{A}_w\, g_0(\mathbf{r}_w,t_j|\mathbf{r}_k,t_l)\, N_T(\mathbf{r}_k,t_l)\,.$$

The exact solution of (47) is given by the series

$$(48)\quad g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) = \sum_{r=0}^{\infty} g_r(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j)$$

and

$$g_r(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) \approx \frac{\mathcal{T}_j}{N_T(\mathbf{r}_w,t_j|\mathbf{r}_k,t_l)} \sum_{v=1}^{n} \mathcal{A}_v\, g_{r-1}(\mathbf{r}_i,t_{j+1}|\mathbf{r}_v,t_j)\, c_T(\mathbf{r}_v,t_j)$$

for $r = 1, 2, \ldots$. For cases where $m \ll f$, that is, if the number of tagged fish recaptured in the interval of time $\mathcal{T}_j$ is

$$N_R(t_{j+1}) \approx \mathcal{T}_j \sum_{i=1}^{n} \mathcal{A}_i\, c_T(\mathbf{r}_i,t_j)\,,$$

the approximation

$$g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) \approx g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) + \frac{\mathcal{T}_j}{N_T(\mathbf{r}_w,t_j|\mathbf{r}_k,t_l)}$$

$$\times \sum_{v=1}^{n} \mathcal{A}_v\, g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_v,t_j)\, c_T(\mathbf{r}_v,t_j)$$

will give accurate results if $N_R < N_T$ but $N_R \not\ll N_T$. As I mentioned in Section 3, if $N_R \ll N_T$, implying that natural death rate is much greater than that due to fishing, then the approximation $g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j) \approx g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_w,t_j)$ is adequate. However, in general, one can proceed by expanding and stop the expansion with the term whose contribution is negligible.

Either the exact or approximate solution for $g$ requires the knowledge of the transition probability density elements $\mathcal{G}_0^* = \{g_0(\mathbf{r}_i,t_{j+1}|\mathbf{r}_v,t_j) : i, v = 1, 2, \ldots, n;\ j = l, l+1, \ldots, m\}$. For tagged fish releases starting in the interval of time $\mathcal{T}_l$ the empirical construction of $g_0$ by (46) yields only $\mathcal{G}_0(\mathbf{r}_k,t_l)$. So it is necessary to calculate the transition probability density elements. This will be done with the property of superposition of probability densities (Property G in Section 3).

The discrete version of the superposition property of probability densities for consecutive time intervals is given by

$$(49)\quad g_0(\mathbf{r}_i,t_{l+2}|\mathbf{r}_k,t_l) \approx \sum_{v=1}^{n} \mathcal{A}_v\, g_0(\mathbf{r}_i,t_{l+2}|\mathbf{r}_v,t_{l+1})\, g_0(\mathbf{r}_v,t_{l+1}|\mathbf{r}_k,t_l)\,.$$

In order to define a completely determined system of equations for the transition probability densities $\mathcal{G}_0^*(\mathcal{T}_{l+1}) = \{g_0(\mathbf{r}_i,t_{l+2}|\mathbf{r}_v,t_{l+1}) : i, v = 1, 2, \ldots, n\}$ from the information of a single tagging experiment, it is necessary to assume that the Green function is spatially translationally invariant. That is, it is necessary to assume that no matter where the tagged fish are released, the results are identical. However, the concept of translational invariance of the Green function is incompatible with the presence of boundaries, for the results of a tagged fish release away from the boundaries, for example, will be different from the results if the release were close to a boundary. Furthermore, fishing effort is not uniform throughout $\mathcal{A}$. Thus, one cannot possibly define a completely determined system of equations for the transition probability densities $\mathcal{G}_0^*(\mathcal{T}_{l+1})$ from the information of a single tagging experiment if the population has a bounded domain or there is an inhomogeneous distribution of fishing death rate. Investigation of (49) leads to the conclusion that at least $n$ tagging experiments are necessary to perform, one per cell, to define a completely determined system of equations. As can be appreciated in (49), if $n$ simultaneous tagged fish releases were done, one per cell, the data at the initial interval of time and all subsequent intervals define completely determined systems of $n$ equations. However, it is not necessary to perform the tagged fish releases simultaneously. To see this, it is best to consider an example.

For simplicity, I will suppose that the release at time $t_l$ is in cell $\mathcal{A}_1$, the release at time $t_{l+1}$ is in cell $\mathcal{A}_2, \ldots,$ and the release at time $t_{l+n-1}$ is in cell $\mathcal{A}_n$. To determine the complete set of transition probability densities for an interval of time, it is necessary that there be as many tagging experiments as there are cells in the domain, one per cell, but it is sufficient that there be only one experiment per interval of time. Some or all can be done simultaneously. With the catch data of each experiment, because I have assumed distinguishable tags, one has the set of sets $\mathcal{C}_T = \{\mathcal{C}_T(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$, that is, the set of catch density rates of the $n$ tagging experiments. Use of $\mathcal{C}$ and $\mathcal{C}_T$ in equation (45) will yield a set of computed catchabilities given by $\mathcal{Q} = \{q(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$. I can use these to compute a set of discrete Green functions $\mathcal{G}_0 = \{\mathcal{G}_0(\mathbf{r}_s,t_{l+s-1}) : s = 1, 2, \ldots, n\}$ as indicated by (46). However, the catchability of the $i$th cell at time $t_j$ should be used for the computation of terms of all $n$ Green functions evaluated in the $i$th cell (i.e., receiver point $\mathbf{r}_i$) at time $t_j$.

Assuming the tagged fish live longer than $[t_l,t_{l+n}]$, the second transition when there is data from all $n$ tagging experiments is during interval of time $\mathcal{T}_{l+n}$. I am arbitrarily using the transitions at $\mathcal{T}_{l+n}$ rather than those at $\mathcal{T}_{l+n-1}$ for the sake of uniformity of notation in the analysis that follows. From (49), for the release of tagged fish in $\mathcal{A}_1$:

$$g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_1,t_l) \approx \sum_{v=1}^{n} \mathcal{A}_v\, g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_v,t_{l+n})\, g_0(\mathbf{r}_v,t_{l+n}|\mathbf{r}_1,t_l)\,.$$

For the release of tagged fish in $\mathcal{A}_2$:

$$g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_2,t_{l+1}) \approx \sum_{v=1}^{n} \mathcal{A}_v\, g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_v,t_{l+n})\, g_0(\mathbf{r}_v,t_{l+n}|\mathbf{r}_2,t_{l+1})$$

$$\vdots$$

and finally, for the release of tagged fish in $\mathcal{A}_n$:

$$g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_n,t_{l+n-1}) \approx \sum_{v=1}^{n} \mathcal{A}_v g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_v,t_{l+n})\, g_0(\mathbf{r}_v,t_{l+n}|\mathbf{r}_n,t_{l+n-1})\,.$$

This is a system of equations of the form

$$\text{(50)}\quad \mathbf{d}_s = \sum_{v=1}^{n} \mathcal{P}_{sv}\, \mathbf{m}_v \text{ for } s = 1, 2, \ldots, n\,.$$

In this equation, identify by comparison with (49)

$$\mathbf{d} = \begin{pmatrix} g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_1,t_l) \\ g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_2,t_{l+1}) \\ \cdot \\ g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_n,t_{l+n-1}) \end{pmatrix}$$

as the data vector,

---

$$\mathcal{P} = \begin{pmatrix} \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n}|\mathbf{r}_1,t_l) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n}|\mathbf{r}_1,t_l) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n}|\mathbf{r}_1,t_l) \\ \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n}|\mathbf{r}_2,t_{l+1}) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n}|\mathbf{r}_2,t_{l+1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n}|\mathbf{r}_2,t_{l+1}) \\ \cdot & \cdot & \cdot & \cdot \\ \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n}|\mathbf{r}_n,t_{l+n-1}) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n}|\mathbf{r}_n,t_{l+n-1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n}|\mathbf{r}_n,t_{l+n-1}) \end{pmatrix}$$

as the $n \times n$ matrix of coefficients, and

$$\mathbf{m} = \begin{pmatrix} g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_1,t_{l+n}) \\ g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_2,t_{l+n}) \\ \cdot \\ g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_n,t_{l+n}) \end{pmatrix}$$

as the model vector. Solving this system of equations for $i = 1, 2, \ldots, n$ gives the set $\mathcal{G}_0^*(\mathcal{T}_{l+n}) = \{g_0(\mathbf{r}_i,t_{l+n+1}|\mathbf{r}_v,t_{l+n}) : i, v = 1, 2, \ldots, n\}$. Following the previous procedure for the next time step (i.e., $\mathcal{T}_{l+n+1}$), solve (50) with

$$\mathbf{d} = \begin{pmatrix} g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_1,t_l) \\ g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_2,t_{l+1}) \\ \cdot \\ g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_n,t_{l+n-1}) \end{pmatrix}$$

as the data vector,

$$\mathcal{P} = \begin{pmatrix} \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n+1}|\mathbf{r}_1,t_l) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n+1}|\mathbf{r}_1,t_l) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n+1}|\mathbf{r}_1,t_l) \\ \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n+1}|\mathbf{r}_2,t_{l+1}) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n+1}|\mathbf{r}_2,t_{l+1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n+1}|\mathbf{r}_2,t_{l+1}) \\ \cdot & \cdot & \cdot & \cdot \\ \mathcal{A}_1 g_0(\mathbf{r}_1,t_{l+n+1}|\mathbf{r}_n,t_{l+n-1}) & \mathcal{A}_2 g_0(\mathbf{r}_2,t_{l+n+1}|\mathbf{r}_n,t_{l+n-1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n,t_{l+n+1}|\mathbf{r}_n,t_{l+n-1}) \end{pmatrix}$$

as the matrix of coefficients, and

$$\mathbf{m} = \begin{pmatrix} g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_1,t_{l+n+1}) \\ g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_2,t_{l+n+1}) \\ \cdot \\ g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_n,t_{l+n+1}) \end{pmatrix}$$

as the model vector. Its solution for $i = 1, 2, \ldots, n$ will yield the set $\mathcal{G}_0^*(\mathcal{T}_{l+n+1}) = \{g_0(\mathbf{r}_i,t_{l+n+2}|\mathbf{r}_v,t_{l+n+1}) : i, v = 1, 2, \ldots, n\}$. Proceeding successively to the last system of equations, which in the interval of time $\mathcal{T}_m$, are given by

$$\mathbf{d} = \begin{pmatrix} g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_1, t_l) \\ g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_2, t_{l+1}) \\ \cdot \\ g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_n, t_{l+n-1}) \end{pmatrix}$$

as the data vector,

$$\mathcal{P} = \begin{pmatrix} \mathcal{A}_1 g_0(\mathbf{r}_1, t_m | \mathbf{r}_1, t_l) & \mathcal{A}_2 g_0(\mathbf{r}_2, t_m | \mathbf{r}_1, t_l) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n, t_m | \mathbf{r}_1, t_l) \\ \mathcal{A}_1 g_0(\mathbf{r}_1, t_m | \mathbf{r}_2, t_{l+1}) & \mathcal{A}_2 g_0(\mathbf{r}_2, t_m | \mathbf{r}_2, t_{l+1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n, t_m | \mathbf{r}_2, t_{l+1}) \\ \cdot & \cdot & \cdot & \cdot \\ \mathcal{A}_1 g_0(\mathbf{r}_1, t_m | \mathbf{r}_n, t_{l+n-1}) & \mathcal{A}_2 g_0(\mathbf{r}_2, t_m | \mathbf{r}_n, t_{l+n-1}) & \cdot & \mathcal{A}_n g_0(\mathbf{r}_n, t_m | \mathbf{r}_n, t_{l+n-1}) \end{pmatrix}$$

as the matrix of coefficients, and

$$\mathbf{m} = \begin{pmatrix} g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_1, t_m) \\ g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_2, t_m) \\ \cdot \\ g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_n, t_m) \end{pmatrix}$$

as the model vector. Solving these for $i = 1, 2, \ldots, n$ will yield the set $\mathcal{G}_0^*(\mathcal{T}_m) = \{g_0(\mathbf{r}_i, t_{m+1} | \mathbf{r}_v, t_m) : i, v = 1, 2, \ldots, n\}$. Any number of numerical techniques can be used to solve (49), or the integral equations of Section 3 for those cases in which an analytical model is chosen (Franklin 1970; Tikhonov and Arsenin 1977; Lavrentev et al. 1986; Parker 1990; Wahba 1990; Press et al. 1992). For noisy data characteristic of fisheries, stable solutions resulting from the inversion of the above systems of equations will have the effect of smoothing the solutions at the expense of resolution. In the end of this process of solving these $m - l - n$ systems of $n$ equations, I will have constructed the set $\mathcal{G}_0^* = \mathcal{G}_0^*(\mathcal{T}_{l+n}) \cup \mathcal{G}_0^*(\mathcal{T}_{l+n+1}) \cup \ldots \cup \mathcal{G}_0^*(\mathcal{T}_m) = \{\mathcal{G}_0^*(\mathcal{T}_j) : j = l + n, l + n + 1, \ldots, m\} = \{g_0(\mathbf{r}_i, t_{j+1} | \mathbf{r}_v, t_j) : i, v = 1, 2, \ldots, n; = l + n, l + n + 1, \ldots, m\}$ necessary to generate the set $\mathcal{G}^* = \{g(\mathbf{r}_i, t_{j+1} | \mathbf{r}_v, t_j) : i, v = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$ of effort-independent transition probability densities by use of (48).

If the population exhibits periodic behavior of period $\mathcal{T} < t_{m+1} - t_{l+n}$, then some of the transitions will be repeated because

$$g_0(\mathbf{r}_i, t_{j+1} | \mathbf{r}_v, t_j) = g_0(\mathbf{r}_i, t_{j+1} + \mathcal{T} | \mathbf{r}_v, t_j + \mathcal{T}) .$$

It is desirable that tagged fish persist in their domain as long as possible but at least for two periods. This means that, in general, some transitions will have more than one value, one for each period that the tagged fish persist in their domain and there is catch of tagged fish. Due to statistical fluctuations, they will be generally unequal and can be averaged.

Natural periods, such as seasonal, are at best approximate. Due to the inherently stochastic nature of fish movement, the approximate repetition of their seasonal movement may be advanced or retarded. However, the transition probability densities may help decide the duration of a period by the consistent lack or peaks of a subset of $\mathcal{G}^*$, the set of effort-independent transition probability densities. For the estimation of periods the set $\mathcal{G}_0^*$ should not be used because some of the periodic nature of its subsets could be due to seasonal application of fishing effort.

Once the set $\mathcal{G}^*$ of effort-independent transition probability density elements for the fish dynamics are determined, they can be used to determine the contribution to the population density due to recruitment. The discrete version of (44) for a single time interval is

$$(51) \quad u(\mathbf{r}_i, t_{j+1}) \approx \frac{c(\mathbf{r}_i, t_{j+1})}{q(\mathbf{r}_i, t_{j+1}\, e(\mathbf{r}_i, t_{j+1})} - \sum_{v=1}^{n} \mathcal{A}_v g(\mathbf{r}_i, t_{j+1} | \mathbf{r}_v, t_j) \times \left[ \frac{1}{f(\mathbf{r}_v, t_j)} - \mathcal{T}_j \right] c(\mathbf{r}_v, t_j) .$$

Because I have sets $\mathcal{C}$, $\mathcal{Q}$, $\mathcal{E}$, and $\mathcal{G}^*$, I can compute with (51) the set $\mathcal{U} = \{u(\mathbf{r}_i, t_{j+1}) : i = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$. To compute the catch density rate $\mathcal{C}' = \{c'(\mathbf{r}_i, t_{j+1}) : i = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$ at a new level of effort density $\mathcal{E}' = \{e'(\mathbf{r}_i, t_{j+1}) : i = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$, the population density at the different level of effort density can be computed with the discretized version of (41) which for a single interval of time $\mathcal{T}_j$ is given by

$$(52) \quad p'(\mathbf{r}_i, t_{j+1}) \approx \sum_{v=1}^{n} \mathcal{A}_v g(\mathbf{r}_i, t_{j+1} | \mathbf{r}_v, t_j) [1 - \mathcal{T}_j q(\mathbf{r}_v, t_j) \times e'(\mathbf{r}_v, t_j)] p(\mathbf{r}_v, t_j) + u(\mathbf{r}_i, t_{j+1})$$

and is applied recursively for $j = l + n, l + n + 1, \ldots, m$. In (52), one assumes that the recruitment density rate does not change significantly at the new level of effort. The catch density rate $\mathcal{C}'$ at the different level of effort density $\mathcal{E}'$ is computed by multiplying the result of (52) by $q(\mathbf{r}_i, t_{j+1}) e'(\mathbf{r}_i, t_{j+1})$.

To summarize the previous example, the following steps must be taken to solve the fisheries interaction problem:

1. Using the set of tagged fish catch density rates of $n$ tagging experiments ($\mathcal{C}_T$) and the set of effort density measurements ($\mathcal{E}$), the set of catchabilities ($\mathcal{Q}$) is constructed by use of (45). There will be one catchability associated with each cell and interval of time where tagged fish are released. If only one tagging experiment is conducted in each cell, then one is forced to assume that the value of each catchability in each cell is time independent.

2. Using the sets $\mathcal{C}_T$, $\mathcal{E}$, and $\mathcal{Q}$, the set of discrete Green functions ($\mathcal{G}_0$) for the $n$ tagging experiments is constructed by use of (46).

3. The set of effort-dependent transition probability densities ($\mathcal{G}_0^*$) is constructed by application of $\mathcal{G}_0$ to (49).

4. The set of effort-independent transition probability densities ($\mathcal{G}^*$) is constructed by application of $\mathcal{G}_0^*$ to (48).

5. With the set of catch density rates of untagged fish ($\mathcal{C}$) and sets $\mathcal{Q}$, $\mathcal{E}$, and $\mathcal{G}^*$, the set of population densities due to recruitment $\mathcal{U}$ is constructed by applying (51).

6. Assuming a new level of effort given by the set $\mathcal{E}' \neq \mathcal{E}$, the resulting catch $\mathcal{C}'$ is computed using $\mathcal{E}'$, $\mathcal{Q}$, and $\mathcal{U}$ in (52) and multiplying the population densities $p'(\mathbf{r}_i,t_{j+1})$ by $f(\mathbf{r}_i,t_{j+1}) = q(\mathbf{r}_i,t_{j+1})e'(\mathbf{r}_i,t_{j+1})$.

The recruitment density rate $\mathcal{R} = \{r(\mathbf{r}_i,t_j) : i = 1, 2, \ldots, n; j = l + n, l + n + 1, \ldots, m\}$ is computed by inversion of the system by inversion of the system of equations

$$u(\mathbf{r}_i,t_{j+1}) \approx \mathcal{T}_j \sum_{\nu=1}^{n} \mathcal{A}_\nu g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_\nu,t_j)\, r(\mathbf{r}_\nu,t_j)\,.$$

which is the discretized field $u(\mathbf{r},t)$ given in (42) for a single time interval $\mathcal{T}_j$. For this system of equations, identify in (50)

$$\mathbf{d} = \begin{pmatrix} u(\mathbf{r}_1,t_{j+1}) \\ u(\mathbf{r}_2,t_{j+1}) \\ \cdot \\ u(\mathbf{r}_n,t_{j+1}) \end{pmatrix}$$

as the data vector,

$$\mathcal{P} = \mathcal{T}_j \begin{pmatrix} \mathcal{A}_1 g(\mathbf{r}_1,t_{j+1}|\mathbf{r}_1,t_j) & \mathcal{A}_2 g(\mathbf{r}_1,t_{j+1}|\mathbf{r}_2,t_j) & \cdot & \mathcal{A}_n g(\mathbf{r}_1,t_{j+1}|\mathbf{r}_n,t_j) \\ \mathcal{A}_1 g(\mathbf{r}_2,t_{j+1}|\mathbf{r}_1,t_j) & \mathcal{A}_2 g(\mathbf{r}_2,t_{j+1}|\mathbf{r}_2,t_j) & \cdot & \mathcal{A}_n g(\mathbf{r}_2,t_{j+1}|\mathbf{r}_n,t_j) \\ \cdot & \cdot & \cdot & \cdot \\ \mathcal{A}_1 g(\mathbf{r}_n,t_{j+1}|\mathbf{r}_1,t_j) & \mathcal{A}_2 g(\mathbf{r}_n,t_{j+1}|\mathbf{r}_2,t_j) & \cdot & \mathcal{A}_n g(\mathbf{r}_n,t_{j+1}|\mathbf{r}_n,t_j) \end{pmatrix}$$

as the $n \times n$ matrix of coefficients, and

$$\mathbf{m} = \begin{pmatrix} r(\mathbf{r}_1,t_j) \\ r(\mathbf{r}_2,t_j) \\ \cdot \\ r(\mathbf{r}_n,t_j) \end{pmatrix}$$

as the model vector. The system of equations must be solved for the $j = l + n, l + n + 1, \ldots, m$ time intervals. However, in order to be able to accurately resolve the population density due to recruitment from the population at large by use of (51), it is essential that the Green function also be a measure of the survivability and movement of the untagged portion of the population. As has been discussed before, this condition is true only if sufficient numbers of tagged fish are released.

Once the recruitment density rate is resolved from the above inversions, I am free to interpret it as a nonlinear function of population density. The same result is achieved by inverting for the recruitment density rate using the corresponding algebraic equations that involve the effort-dependent Green function.

## 6. Estimation of the Field Equation Coefficients

In this section, I will show how to calculate the coefficients of the field equation using tagged fish data. This method is a modification of an analytical technique that is used to construct solutions of stochastic differential equations from Fokker–Planck equations (Okubo 1980; Zwillinger 1989) by the use of moments. The modification developed here will lead to the identification of a closed-form expression for the transition probability densities for small intervals of time. I will show that because the moment method is unable to resolve the divergence of the drift velocity and the Laplacian of the diffusivity, I am left with two alternative models that do equally well interpreting the data.

I assume that I have resolved a smooth set $\mathcal{G}^*$ of the effort-independent transition probability density elements. However, this analysis can also be done with $\mathcal{G}_0^*$. The Green function of the dynamics the population follows is given by

$$(53)\quad \left[\frac{\partial}{\partial t} + \mathcal{L}\right] g(\mathbf{r},t|\mathbf{r}',t') = \delta(\mathbf{r} - \mathbf{r}')\,\delta(t - t')$$

in the space–time domain $\mathbf{r} \in \mathcal{A}$, where operator $\mathcal{L}$ is

$$\mathcal{L} = b_0(\mathbf{r},t) + \mathbf{b}_1(\mathbf{r},t) \cdot \nabla + b_2(\mathbf{r},t)\,\Delta$$

subject to causality condition

$$g(\mathbf{r},t|\mathbf{r}',t') = 0 \text{ if } t < t'$$

and boundary conditions

$$g(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_A$ and

$$\mathbf{n}_\perp \cdot \nabla g(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_R$. The only difference between this field equation and (7) is that here, $b_0(\mathbf{r},t) = m(\mathbf{r},t) + \nabla \cdot \mathbf{v}(\mathbf{r},t)$.

The even moments of $g$ are found by multiplying (53) by $r^{2N}$ and integrating over space $\mathcal{A}$ in the interval of time $\mathcal{T}_{-\infty} = [-\infty,t]$. Once these integrations are performed, space–time discretization of the resulting equation for a single interval of time and invoking the initial property of $g$, for a source in cell $\mathcal{A}_k$ in the interval of time $\mathcal{T}_j$, leads to the equation for the $2N$th moment of $g$ (Appendix C) given by

(54) $$r_k^{2N} m(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}\,\mathbf{r}_k \cdot \mathbf{v}(\mathbf{r}_k,t_j) - 4N^2 r_k^{2N-2} k(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}\mathbf{r}_k \cdot \nabla_k k(\mathbf{r}_k,t_j) = \frac{1}{\mathcal{T}_j}\left[r_k^{2N} - \langle r_i^{2N}\rangle(t_{j+1}|\mathbf{r}_k,t_j)\right]$$

for $\mathbf{r}_k \notin \partial\mathcal{A}$, where

$$\langle r_i^{2N}\rangle(t_{j+1}|\mathbf{r}_k,t_j) = \sum_{i=1}^{n} \mathcal{A}_i\, r_i^{2N}\, g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j)\,.$$

The odd moments are calculated by going through the same process after multiplication of the field equation for $g$ by $r^{2N}\mathbf{r}$. The result is (Appendix C)

(55) $$r_k^{2N}\mathbf{r}_k m(\mathbf{r}_k,t_j) - r_k^{2N}\mathbf{v}(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}\mathbf{r}_k[\mathbf{r}_k \cdot \mathbf{v}(\mathbf{r}_k,t_j)] - 4N(N+1)\, r_k^{2N-2}\mathbf{r}_k\, k(\mathbf{r}_k,t_j)$$
$$- r_k^{2N}\nabla_k k(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}\mathbf{r}_k[\mathbf{r}_k \cdot \nabla_k k(\mathbf{r}_k,t_j)] = \frac{1}{\mathcal{T}_j}\left[r_k^{2N}\mathbf{r}_k - \langle r_i^{2N}\mathbf{r}_i\rangle(t_{j+1}|\mathbf{r}_k,t_j)\right]$$

for $\mathbf{r}_k \notin \partial\mathcal{A}$, where

$$\langle r_i^{2N}\,\mathbf{r}_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) = \sum_{i=1}^{n} \mathcal{A}_i\, r_i^{2N}\mathbf{r}_i\, g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j)\,.$$

Because data are collected only at the points that are interior to $\mathcal{A}$, I will consider only those source points $\mathbf{r}_k \notin \partial\mathcal{A}$. For points $\mathbf{r}_k \notin \partial\mathcal{A}$, corrections are necessary (Appendix C). However, for the purposes of this work, they are unnecessary.

With the moments of the transition probability densities for a single interval of time $\mathcal{T}_j$, it is not possible to estimate the values of $\nabla_k \cdot \mathbf{v}(\mathbf{r}_k,t_j)$ and $\triangle_k k(\mathbf{r}_k,t_j)$ in cell $\mathcal{A}_k$. As can be appreciated in (54) and (55), the even- and odd-moment equations are independent of these parameters. For the remaining parameters, $\{m(\mathbf{r}_k,t_j), \mathbf{v}(\mathbf{r}_k,t_j), k(\mathbf{r}_k,t_j), \nabla_k k(\mathbf{r}_k,t_j)\}$, there are restrictions to their resolvablility. To see this, construct from the moment equations (54) and (55) a completely determined system of linear equations. Because the even-moment equations are scalar and the odd moments generate two component vector equations, it is only necessary to compute up to the third order moment. It leads to a system of linear algebraic equations

$$\mathbf{d}_s = \sum_{v=1}^{6} \mathcal{P}_{sv}\,\mathbf{m}_v \qquad s = 1, 2, \ldots, 6$$

with

$$\mathbf{d} = \frac{1}{\mathcal{T}_j}\begin{pmatrix} 1 - \langle 1\rangle(t_{j+1}|\mathbf{r}_k,t_j) \\ x_k - \langle x_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) \\ y_k - \langle y_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) \\ r_k^2 - \langle r_i^2\rangle(t_{j+1}|\mathbf{r}_k,t_j) \\ r_k^2 x_k - \langle r_i^2 x_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) \\ r_k^2 y_k - \langle r_i^2 y_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) \end{pmatrix}$$

as the data vector

$$\mathbf{m} = \begin{pmatrix} m(\mathbf{r}_k,t_j) \\ v_x(\mathbf{r}_k,t_j) \\ v_y(\mathbf{r}_k,t_j) \\ k(\mathbf{r}_k,t_j) \\ \partial k(\mathbf{r}_k,t_j)/\partial x_k \\ \partial k(\mathbf{r}_k,t_j)/\partial y_k \end{pmatrix}$$

as the model vector, and

$$\mathcal{P} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ x_k & -1 & 0 & 0 & -1 & 0 \\ y_k & 0 & -1 & 0 & 0 & -1 \\ r_k^2 & -2x_k & -2y_k & -4 & -2x_k & -2y_k \\ r_k^2 x_k & -r_k^2 - 2x_k^2 & -2x_k y_k & -8x & -r_k^2 - 2x_k^2 & -2\,x_k y_k \\ r_k^2 y_k & -2x_k y_k & -r_k^2 - 2y_k^2 & -8y & -2x_k y_k & -r_k^2 - 2y_k^2 \end{pmatrix}$$

as the matrix of coefficients. The matrix of coefficients $\mathcal{P}$ has $det\mathcal{P} = 0$. Therefore, the equations are not linearly independent. Keeping natural death rate and diffusion, there are two alternatives regarding the fields $\mathbf{v}$ and $\nabla_k k$ within cell $\mathcal{A}_k$ during the interval of time $\mathcal{T}_j$: either $\mathbf{v}(\mathbf{r}_k, t_j)$ is constant and $\nabla_k k(\mathbf{r}_k,t_j) = 0$ or $\mathbf{v}(\mathbf{r}_k,t_j) = 0$ and $\nabla_k k(\mathbf{r}_k,t_j)$ is constant. In either case, it is only necessary to consider up to the second moment to define a completely determined system of equations. The matrix of

coefficients given by

$$\mathcal{P} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ x_k & -1 & 0 & 0 \\ y_k & 0 & -1 & 0 \\ r_k^2 & -2x_k & -2y_k & -4 \end{pmatrix},$$

which has a determinant given by $det\mathcal{P} = -4$, and the data vector given by

$$\mathbf{d} = \frac{1}{\mathcal{T}_j} \begin{pmatrix} 1 - \langle\, 1 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) \\ x_k - \langle\, x_i \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) \\ y_k - \langle\, y_i \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) \\ r_k^2 - \langle\, r_i^2 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) \end{pmatrix}$$

are sufficient to define uniquely

$$\mathbf{m} = \begin{pmatrix} m(\mathbf{r}_k,t_j) \\ v_x(\mathbf{r}_k,t_j) \\ v_y(\mathbf{r}_k,t_j) \\ k(\mathbf{r}_k,t_j) \end{pmatrix}$$

in the first alternative or

$$\mathbf{m} = \begin{pmatrix} m(\mathbf{r}_k,t_j) \\ \partial k(\mathbf{r}_k,t_j)/\partial x_k \\ \partial k(\mathbf{r}_k,t_j)/\partial y_k \\ k(\mathbf{r}_k,t_j) \end{pmatrix}$$

in the second alternative. Because the moment method cannot resolve the derivatives $\nabla_k \cdot \mathbf{v}(\mathbf{r}_k,t_j)$ and $\Delta_k k(\mathbf{r}_k,t_j)$, either of the alternatives above will interpret the data equally well. In other words, because the divergence of $\mathbf{v}$ and the Laplacian of $k$ are unresolvable by the moment method, the inverse will interpret the data equally well if $\mathbf{v} = b(t_j)$ and $k = a(t_j)$ or $\mathbf{v} = 0$ and $k = a(t_j) - b(t_j)\mathbf{r}_k/2$. This does not mean that the entire domain of the population has $\{m,\mathbf{v},k\}$ or $\{m,\nabla_k k\}$ that are independent of position. It means that within each cell of the domain, each, in principle, arbitrarily small, the resolved coefficients $\{m,\mathbf{v},k\}$ or $\{m,\nabla_k k\}$ are constant within an interval of time.

The solution to the system of equations for the case of the first alternative is

$$m(\mathbf{r}_k,t_j) = \frac{1}{\mathcal{T}_j}\Big[1 - \langle\, 1 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j)\Big]$$

$$\mathbf{v}(\mathbf{r}_k,t_j) = \frac{1}{\mathcal{T}_j}\Big[\langle\, \mathbf{r}_i \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) - \mathbf{r}_k \langle\, 1 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j)\Big]$$

$$k(\mathbf{r}_k,t_j) = \frac{1}{4\,\mathcal{T}_j}\Big[\langle\, r_i^2 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) - 2\mathbf{r}_k \cdot \langle\, \mathbf{r}_i \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j) + r_k^2 \langle\, 1 \,\rangle\, (t_{j+1}|\mathbf{r}_k,t_j)\Big].$$

These are the same results, linearized in $\mathcal{T}_j$, that the discrete transition probability density

$$(56) \quad g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j) = \exp\,[-\mathcal{T}_j\, m(\mathbf{r}_k,t_j)]\; \mathcal{N}\,(\mathbf{r}_k + \mathcal{T}_j\, \mathbf{v}(\mathbf{r}_k,t_j),\sigma^2)$$

where

$$\sigma^2 = 2\,\mathcal{T}_j\, k(\mathbf{r}_k,t_j)$$

(see equation (20)) yields for the unbounded domain when its coefficients are calculated using moments. In fact, (56) yields the moments of $g$ of any order in the interval of time $\mathcal{T}_j$, but those derived here (Appendix C) are linearized in $\mathcal{T}_j$ due to the discretization of the field equation necessary for the derivation. The fact that the moment equations of this section are correct for either the unbounded domain, or the domains bounded by absorbing and/or reflecting boundaries if $\mathbf{r}_k \notin \partial\mathcal{A}$ (Appendix C), leads to the expectation that the closed-form transition probability (56) is also correct for the bounded domain of this work. Because $\mathcal{T}_j$ is small, the transition probability densities are very nearly Dirac delta functions, so for points $\mathbf{r}_i$ not near $\mathbf{r}_k$ the distributions are nearly vanishing.

Finally, transforming (56) with the transformation $m(\mathbf{r}_k,t_j) \rightarrow m(\mathbf{r}_k,t_j) + f(\mathbf{r}_k,t_j)$ yields the closed-form expression for the effort-dependent transition probability densities. These results, coupled with the discrete equations of Section 5, simplify considerably numerical analysis and simulations for an interval of time of arbitrary length. This method should be applicable to problems whose field equation has a Green function with a probabilistic interpretation: Fokker–Planck equations, but more generally, any linear field equation with at most a first-order time derivative but spatial derivatives of arbitrary order. As can be seen by comparing (20) with (56), it is only necessary to find the Green function associated with the field equation that is invariant under space–time translations, and then expressions for small intervals of time for the discrete transition probability densities associated with the corresponding field equation that is not invariant under space–time translations are constructed by inspection. In general, it is only possible to construct exact closed-form expressions for the transition probability densities if the differential operator has spacial derivatives no higher than fourth order.

## 7. Summary

When members of a population are released in an area small compared with their domain and over an interval of time short compared with the length of time they take to disperse throughout their domain, the pattern of movement approximates a point-source solution (or Green function) of the underlying population dynamics. The Green functions, one for each release, can be empirically determined with the mark and recapture data of tagged members of the population with distinguishable tags. The assumed field equations for the population dynamics were inverted to represent the solution in terms of integral equations for the tagged and untagged portions of the population.

Because for a fish population it is necessary to use catch per unit effort of tagged fish to construct the Green functions empirically, the resulting point-source solutions are effort dependent and, therefore, not practical for a fishery interaction problem where it is desired to determine the catch resulting from a different level of effort, the field equations were reformulated such that the term containing effort was redefined as an inhomo-

geneous term of the field equation. This leads to a method of constructing the effort-independent Green function from knowledge of the effort-dependent one.

Discretization of the integral equations was achieved by defining non overlapping cells of arbitrary shape in the neighborhood of each point where data were collected. The integral equations of the theory, upon discretization, result as systems of coupled algebraic equations which for a single interval of time coincide with a Markovian formulation of the exchange of members of the population between the cells of the discretized space.

For every tagging experiment, it is possible to calculate only one catchability at the cell and time of release. However, to be able to determine approximately a complete effort-independent Green function from knowledge of the effort-dependent one in a bounded population domain, it is required that there be as many tagging experiments as there are cells defined in the domain.

The Green functions were interpreted as the tagged fish probability density of surviving movement to the receiver space–time coordinates from the release coordinates. If sufficiently large numbers of tagged fish are released, then the tagged fish dynamics are representative of the population. Both the effort-dependent and effort-independent Green fuctions can be decomposed into transition probability densities for a single interval of time. The transition probability densities can be used to determine the recruitment density rate which, once resolved, can be freely interpreted as a nonlinear function of the population density. The moments of the transition probability densities lead to the evaluation of model parameters and the identification of the closed-form transition probability densities.

## Acknowledgements

Fruitful conversations were held with Alain Fonteneau, Nancy Lo, and Patrick Tomlinson. I also wish to thank Salah Baouendi, Richard Deriso, Hector Echavarría, and Linda Rothschild for discussions, commentaries, and suggestions. Ian Fletcher and Geneva Simmons provided valuable editorial suggestions. David Fournier, Don Ludwig, and Marc Mangel, who reviewed the original manuscript, provided valuable editorial and technical commentaries. In particular, Pierre Kleiber's support of the research, constant availability for discussions while in residence at the SWFSC, and asking of pertinent questions that influenced the research are very gratefully acknowledged.

## References

BAYLIFF, W.H. 1979. Migration of yellowfin tuna in the eastern Pacific Ocean as determined from tagging experiments initiated during 1968–1974. Inter-Am. Trop. Tuna Comm. Bull. 17: 445–506.

BJORKEN, J.D., AND S.D. DRELL. 1964. Relativistic quantum mechanics. McGraw-Hill Book Co., New York, N.Y. 300 p.

BUTKOV, E. 1968. Mathematical physics. Addison-Wesley Publishing Co., Reading, Mass. 724 p.

BYRON, F.W. JR., AND R.W. FULLER. 1970. Mathematics of classical and quantum mechanics, v. II. Addison-Wesley Publishing Co., Reading, Mass. p. 305–659.

COURANT, R., AND D. HILBERT. 1953. Methods of mathematical physics, v. II. Interscience, New York, N.Y. 830 p.

FEYNMAN, R.P. 1949. The theory of positrons. Phys. Rev. 16: 749–759.

FEYNMAN, R.P., AND A.R. HIBBS. 1965. Quantum mechanics and path integrals. McGraw-Hill Book Co., New York, N.Y. 357 p.

FRANKLIN, J.N. 1970. Well-posed stochastic extensions of ill-posed linear problems. J. Math. Anal. Appl. 31: 682–716.

GARABEDIAN, P.R. 1964. Partial differential equations. John Wiley & Sons, Inc., New York, N.Y. 672 p.

GARDINER, C.W. 1990. Handbook of stochastic methods. Springer-Verlag, Berlin, Germany. 442 p.

GILBARG, D., AND N.S. TRUDINGER. 1979. Elliptic partial differential equations of second order. Springer-Verlag, Berlin, Germany. 401 p.

HILBORN, R. 1990. Determination of fish movement patterns from tag recoveries using maximum likelihood estimators. Can. J. Fish. Aquat. Sci. 47: 635–643.

HUNTER, J.R., A.W. ARGUE, W.H. BAYLIFF, A.E. DIZON, A. FONTENEAU, D. GOODMAN, AND G.R. SECKEL. 1986. The dynamics of tuna movements: an evaluation of past and future research. FAO Fish. Tech. Pap. No. 277: 78 p.

ISHII, T. 1979. Attempt to estimate migration of a fish population with survival parameters from tagging experiment data by the simulation method. Invest Pesq. 43: 301–317.

KLEIBER, P., A.W. ARGUE, AND R.E. KEARNEY. 1987. Assessment of skipjack (*Katsuwonus pelamis*) resources by estimating standing stock and components of population turnover from tagging data. Can. J. Fish. Aquat. Sci. 44: 1122–1134.

LAVRENTEV, M.M., V.G. ROMANOV, AND S.P. SHISHATSKII. 1986. Ill-posed problems of mathematical physics. American Mathematical Society Translations of mathematical monographs, Vol. 64. Providence, R.I. 290 p.

LIGHTHILL, M.J. 1964. Introduction to Fourier analysis and generalized functions. Cambridge, London. 79 p.

MORSE, P.M., AND H. FESHBACK. 1953. Methods of theoretical physics. Vol. I. McGraw-Hill Book Co., New York, N.Y. 997 p.

MURRAY, J.D. 1989. Mathematical biology (Biomathematics v. 19). Springer-Verlag, Berlin, Germany. 767 p.

OKUBO, A. 1980. Diffusion and ecological problems: mathematical models (Biomathematics v. 10). Springer-Verlag, Berlin, Germany. 254 p.

PARKER, R.L. 1990. Geophysical inverse theory. Institute for Geophysics and Planetary Physics, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, Calif. 244 p.

PRESS, W.H., B.P. FLANNERY, S.A. TEUKOLSKY, AND W.T. VETTERLING. 1992. Numerical recipes 2nd ed. Cambridge University Press, Cambridge. 963 p.

RICKER, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

ROYDEN, H.L. 1968. Real analysis. Macmillan Co. London, Great Britain. 349 p.

SALVADÓ, C.A.M. 1994. Discrete population field theory for tag analysis and fishery modeling. Proceedings of the FAO Expert Consultation on Interactions of Pacific Ocean Tuna Fisheries. (In press).

SIBERT, J.R. 1984. A two fishery tag attrition model for the analysis of mortality, recruitment, and fishery interaction. Tech. Rep. No. 13, Tuna and Billfish Assessment Program, South Pacific Commission, Noumea, New Caledonia. 27 p.

SYMONS, P.E.K., P.M. MIYAKE, AND G.T. SAKAGAWA [ED.] 1986. Proceedings of the ICCAT Conference on the International Skipjack Year Program. International Commision for the Conservation of Atlantic Tunas, Madrid. 388 p.

TIKHONOV, A.N., AND V.Y. ARSENIN. 1977. Solutions to ill-posed problems. John Wiley & Sons, Inc., New York, N.Y. 258 p.

TOLSTOV, G.P. 1962. Fourier series. Prentice Hall, Englewood Cliffs, N.J. 336 p.

WAHBA, G. 1990. Spline models for observational data. SIAM. CBMS-NSF Conference Series in Applied Mathematics. 169 p.

ZWILLINGER, D. 1989. Handbook of differential equations. Academic Press, New York, N.Y. 673 p.

## Appendix A. Representation Theorem for the Population Density

The purpose of this appendix is to derive an integral representation for the population density. I will show that if on the boundary of the population domain the population density satisfies either homogeneous Dirichlet of Neumann conditions, the integral representation for the solution does not contain a boundary integral. The proof is not intended to be rigorous, but follows mathematical procedures well established and elucidated in the literature (Courant and Hilbert 1953; Morse and Feshbach 1953; Garabedian 1964; Gilbarg and Trudinger 1979).

Let the domain of the population $\mathcal{A} \in \mathbf{R}^2$ be bounded by $\partial\mathcal{A} \in \mathbf{R}$. Let $\mathbf{r} = x\mathbf{i} + y\mathbf{j} \in \mathcal{A}$ be the receiver or measuring point with respect to an arbitrarily chosen fixed coordinate system where $\mathbf{i}$ and $\mathbf{j}$ are, respectively, the unit vectors pointing in the $+x$ and $+y$ directions. Let time $t$ be the receiver time. At time $t_I$, there is an initial population whose subsequent dynamics I wish to model. I assume that the dynamics of the population are correctly given by (7):

(A1) $$\left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] p(\mathbf{r},t) = r(\mathbf{r},t)$$

where

(A2) $$\mathcal{L}_0\, p(\mathbf{r},t) = b_0(\mathbf{r},t)\, p(\mathbf{r},t) + \mathbf{b}_1(\mathbf{r},t) \cdot \nabla\, p(\mathbf{r},t) + b_2(\mathbf{r},t)\, \Delta\, p(\mathbf{r},t)\,.$$

In equation (A2) the coefficients $\{b_0,\mathbf{b}_1,b_2\}$ are related to the coefficients $\{z,\mathbf{v},k\}$ by

$$b_0(\mathbf{r},t) = z(\mathbf{r},t) + \nabla \cdot \mathbf{v}(\mathbf{r},t)$$

(A3) $$\mathbf{b}_1(\mathbf{r},t) = \mathbf{v}(\mathbf{r},t) - \nabla k(\mathbf{r},t)$$

$$b_2(\mathbf{r},t) = -k(\mathbf{r},t)$$

where the derivatives $\nabla\cdot\mathbf{v}(\mathbf{r},t)$ and $\Delta k(\mathbf{r},t)$ are finite.

The general problem for the population density can be formulated as follows: the population density $p(\mathbf{r},t)$ satisfies the inhomogeneous field equation (A1) in the space–time domain $\mathbf{r} \in \mathcal{A}$, $t \geq t_I$, subject to initial condition

(A4) $$p(\mathbf{r},t_I) = p_{in}(\mathbf{r})$$

in the domain $\mathbf{r} \in \mathcal{A}$. I wish to determine boundary conditions that can be imposed on $\partial\mathcal{A}$ that leads to a unique representation of the solution for $p(\mathbf{r},t)$.

Defining $\mathbf{r}' = x'\mathbf{i} + y'\mathbf{j} \in \mathcal{A}$ as the source point and $t'$ as the source time, the Green function $g_0(\mathbf{r},t|\mathbf{r}',t')$ that is associated with field equation (A1) satisfies field equation

(A5) $$\left[\frac{\partial}{\partial t} + \mathcal{L}_0\right] g_0(\mathbf{r},t|\mathbf{r}',t') = \delta(\mathbf{r}-\mathbf{r}')\,\delta(t-t')$$

in the domain $\mathbf{r} \in \mathcal{A}$ and the causality condition

$$g_0(\mathbf{r},t|\mathbf{r}',t') = 0 \text{ if } t < t'$$

in the domain $\mathbf{r} \in \mathcal{A}$. The key to constructing an integral representation of the solution of an initial-boundary value problem for a field is the ability to construct a bilinear function of the field and a Green function associated with it. Because the differential operator associated with field equation (A5) is not self-adjoint, I must consider the adjoint field equation

(A6) $$\left[-\frac{\partial}{\partial t} + \mathcal{L}_0^*\right] g_0^*(\mathbf{r},t|\mathbf{r}'',t'') = \delta(\mathbf{r}-\mathbf{r}'')\,\delta(t-t'')$$

in the domain $\mathbf{r} \in \mathcal{A}$, where

$$\mathcal{L}_0^*\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'') = b_0(\mathbf{r},t)\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'') - \nabla \cdot [\mathbf{b}_1(\mathbf{r},t)\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'')] + \Delta[b_2(\mathbf{r},t)\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'')]$$

subject to the causality condition

$$g_0^*(\mathbf{r},t|\mathbf{r}'',t'') = 0 \text{ if } t > t'\,.$$

By definition of the field equation that $g_0^*(\mathbf{r},t|\mathbf{r}',t')$ satisfies, it is the function adjoint to Green function $g_0(\mathbf{r},t|\mathbf{r}',t')$.

Multiplying field equation (A5) by $g_0^*(\mathbf{r},t|\mathbf{r}'',t'')$ and (A6) by $g_0(\mathbf{r},t|\mathbf{r}',t')$, subtracting the former resulting equation from the latter resulting equation, and integrating over the interval of time $[-\infty,\infty]$ and over $\mathcal{A}$ leads, by application of the Divergence Theorem, to

$$g_0^*(\mathbf{r}',t'|\mathbf{r}'',t'') - g_0(\mathbf{r}'',t''|\mathbf{r}',t') = \int_{t'}^{t''} dt \oint_{\partial\mathcal{A}} dl\, \mathbf{n}_\perp \cdot \mathbf{P}\,[g_0^*(\mathbf{r},t|\mathbf{r}'',t'')|g_0(\mathbf{r},t|\mathbf{r}',t')]\,.$$

The bilinear function $\mathbf{P}[g_0^*(\mathbf{r},t|\mathbf{r}'',t'')|g_0(\mathbf{r},t|\mathbf{r}',t')]$ is given by

(A7) $$\mathbf{P}[g_o^*(\mathbf{r},t|\mathbf{r}'',t'')|g_0(\mathbf{r},t|\mathbf{r}',t')] = -g_0(\mathbf{r},t|\mathbf{r}',t')\,\mathbf{b}_1(\mathbf{r},t) \times g_0^*(\mathbf{r},t|\mathbf{r}'',t'') + g_0(\mathbf{r},t|\mathbf{r}',t')\,\nabla\,[b_2(\mathbf{r},t)\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'')] - b_2(\mathbf{r},t)\, g_0^*(\mathbf{r},t|\mathbf{r}'',t'')\,\nabla\, g_0(\mathbf{r},t|\mathbf{r}',t') + \mathbf{C}$$

where $\mathbf{C}$ is an arbitrary constant. In order that between $g_0$ and its adjoint $g_0^*$ there be the simple reciprocity relation

(A8) $$g_0^*(\mathbf{r}',t'|\mathbf{r}'',t'') = g_0(\mathbf{r}'',t''|\mathbf{r}',t')\,,$$

it is sufficient that

(A9) $$\mathbf{n}_\perp \cdot \mathbf{P}[g_0^*(\mathbf{r},t|\mathbf{r}'',t'')|g_0(\mathbf{r},t|\mathbf{r}',t')] = 0\,.$$

However, because I also demand uniqueness of the solution, it must vanish in such a way that the conditions imposed that lead to (A9) specify the behavior of both $g$ and its adjoint. Not doing so leads to a solution that is not unique. For example, specifying that $g_0(\mathbf{r},t|\mathbf{r}',t')$ satisfy homogeneous Cauchy conditions (i.e., $g_0(\mathbf{r},t|\mathbf{r}',t') = 0$ and $\mathbf{n}_\perp\cdot\nabla g_0(\mathbf{r},t|\mathbf{r}',t') = 0$ for $\mathbf{r} \in \partial\mathcal{A}$ satisfies (A9) but leaves $g_0^*(\mathbf{r},t|\mathbf{r}'',t'')$ arbitrary. However, the boundary conditions

(A10) $$g_0(\mathbf{r},t|\,\mathbf{r}',t') = 0$$

and

(A11) $$g_0^*(\mathbf{r},t|\,\mathbf{r}',t') = 0$$

or, if $\mathbf{n}_\perp \cdot \mathbf{v}(\mathbf{r},t) = 0$

(A12) $$\mathbf{n}_\perp \cdot \nabla\, g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

and

(A13) $$\mathbf{n}_\perp \cdot \nabla\, g_0^*(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r} \in \partial\mathcal{A}$ result in $g_0$ and $g_0^*$ uniquely specified, and such that the reciprocity relation (A8) holds

Now, as a consequence of (A8), $g_0(\mathbf{r},t|\mathbf{r}',t')$ satisfies the field equation

$$\left[-\frac{\partial}{\partial t'} + \mathcal{L}_0'^*\right] g_0(\mathbf{r},t|\mathbf{r}',t') = \delta(\mathbf{r} - \mathbf{r}')\,\delta(t - t')$$

in the domain $\mathbf{r}' \in \mathcal{A}$, where $\mathcal{L}_0'^*$ is operator $\mathcal{L}_0^*$ under the change of variables $(\mathbf{r},t) \to (\mathbf{r}',t')$. Therefore, the representation of the solution that satisfies field equation (A1) and initial conditions (A4) is given by

$$\text{(A14)}\quad p(\mathbf{r},t) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + \int_{\mathcal{T}_t} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, r(\mathbf{r}',t') + \int_{\mathcal{T}_t} dt' \oint_{\partial\mathcal{A}} dl'\, \mathbf{n}_\perp \cdot \mathbf{P}[g_0(\mathbf{r},t|\mathbf{r}',t'),p(\mathbf{r}',t')]$$

where the integral with respect to time is in the domain $\mathcal{T}_t$ by virtue of the initial condition and causality. In (A14) the bilinear function $\mathbf{P}[g_0(\mathbf{r},t|\mathbf{r}',t'),p(\mathbf{r}',t')]$ is given by

$$\text{(A15)}\quad \mathbf{P}[g_0(\mathbf{r},t|\mathbf{r}',t'),p(\mathbf{r}',t')] = -p(\mathbf{r}',t')\mathbf{b}_1(\mathbf{r}',t') \times g_0(\mathbf{r},t|\mathbf{r}',t') + p(\mathbf{r}',t')\,\nabla'[b_2(\mathbf{r}',t')\, g_0(\mathbf{r},t|\mathbf{r}',t')] - b_2(\mathbf{r}',t')\, g_0(\mathbf{r},t|\mathbf{r}',t')\,\nabla' p(\mathbf{r}',t') + \mathbf{C}$$

where $\mathbf{C}$ is an arbitrary constant. Furthermore, because (A8) holds due to boundary conditions (A10) and (A11) or (A12) and (A13), it follows that $g_0(\mathbf{r},t|\mathbf{r}',t')$ satisfies boundary condition

$$\text{(A16)}\quad g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

or

$$\text{(A17)}\quad \mathbf{n}_\perp \cdot \nabla' g_0(\mathbf{r},t|\mathbf{r}',t') = 0$$

for $\mathbf{r}' \in \partial\mathcal{A}$, and therefore,

$$\text{(A18)}\quad p(\mathbf{r},t) = \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t_T)\, p_{in}(\mathbf{r}') + \int_{\mathcal{T}_t} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g_0(\mathbf{r},t|\mathbf{r}',t')\, r(\mathbf{r}',t')$$

is a unique solution of (A1) subject to initial condition (A4) and homogeneous Dirichlet boundary conditions

$$\text{(A19)}\quad p(\mathbf{r},t) = 0$$

or homogeneous Neumann conditions

$$\text{(A20)}\quad \mathbf{n}_\perp \cdot \nabla p(\mathbf{r},t) = 0$$

in the domain $\mathbf{r} \in \partial\mathcal{A}$.

Assume now that the boundary of $\mathcal{A}$ is composed of segments, denoted $\partial\mathcal{A}_A$, on which homogeneous Dirichlet conditions are satisfied, and the remainder of $\partial\mathcal{A}$, denoted $\partial\mathcal{A}_R$, on which the homogeneous Neumann conditions (A20) are satisfied. Proceeding in the same manner as above, the following conclusion can be made: (A18) uniquely satisfies field equation (A1), initial condition (A4), and boundary conditions

$$p(\mathbf{r},t) = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_A$ and

$$\mathbf{n}_\perp \cdot \nabla p(\mathbf{r},t) = 0$$

for $\mathbf{r} \in \partial\mathcal{A}_R$ if $\mathbf{n}_\perp \cdot \mathbf{v}(\mathbf{r},t) = 0$.

## Appendix B. Convergence Condition of the Neumann Expansion

Here, I shall show that the expansion given in (38) converges if the number of tagged fish recovered during an interval of time is less than the number of tagged fish available at the beginning of the interval. To see this, consider the perturbation expansion given in (38):

$$\text{(B1)}\quad g(\mathbf{r},t|\mathbf{r}_T,t_T) = \sum_{i=0}^{\infty} g_i(\mathbf{r},t|\mathbf{r}_T,t_T)$$

where

$$g_i(\mathbf{r},t|\mathbf{r}_T,t_T) = \frac{1}{N_T(\mathbf{r}_T,t_T)} \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g_{i-1}(\mathbf{r},t|\mathbf{r}',t')\, c_T(\mathbf{r}',t')$$

for $i = 1, 2, \ldots$. Presume now that the maximum number of tagged fish recaptured at any one time is $N_M$. Then, I can state the following:

$$c_T(\mathbf{r}',t') \le \frac{N_R}{\mathcal{A}\mathcal{T}_T^2}$$

where $N_R \le N_M\mathcal{T}_T$ is the number of tagged fish recovered in the interval of time $\mathcal{T}_T$. It follows from the probabilistic property of $g_0$ (Property E in Section 3) that there exists a constant $0 \le C \le 1$ such that

$$\int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2r'\, g_0(\mathbf{r},t|\mathbf{r}',t') \le C\mathcal{T}_T.$$

By making use of the first inequality, the iteration relation can be written

$$g_i(\mathbf{r},t|\mathbf{r}_T,t_T) \le \left(\frac{N_R}{N_T(\mathbf{r}_T,t_T)\mathcal{A}}\right) \frac{1}{\mathcal{T}_T^2} \times \int_{\mathcal{T}_T} dt' \int_{\mathcal{A}} d^2\mathbf{r}'\, g_{i-1}(\mathbf{r},t|\mathbf{r}',t')$$

for $i = 1, 2, \ldots$, and from which I can compute all terms of the iteration:

$$g_1(\mathbf{r},t|\mathbf{r}_T,t_T) \le \left(\frac{C}{\mathcal{A}\,\mathcal{T}_T}\right)\left(\frac{N_R}{N_T(\mathbf{r}_T,t_T)}\right)$$

$$g_2(\mathbf{r},t|\mathbf{r}_T,t_T) \le \left(\frac{C}{\mathcal{A}\,\mathcal{T}_T}\right)\left(\frac{N_R}{N_T(\mathbf{r}_T,t_T)}\right)^2$$

$$g_N(\mathbf{r},t|\mathbf{r}_T,t_T) \le \left(\frac{C}{\mathcal{A}\,\mathcal{T}_T}\right)\left(\frac{N_R}{N_T(\mathbf{r}_T,t_T)}\right)^N$$

Performing the ratio test

$$\lim_{N\to\infty}\left|\frac{g_{N+1}(\mathbf{r},t|\mathbf{r}_T,t_T)}{g_N(\mathbf{r},t|\mathbf{r}_T,t_T)}\right| = \frac{N_R}{N_T(\mathbf{r}_T,t_T)},$$

which is less than 1 if the number of tagged fish recovered is less than the number of tagged fish released, and therefore, the series converges under that condition.

## Appendix C. Moment Equations of the Green Function

In this appendix, I will find expressions for determining the coefficients of the population field equation by computing the moments of the Green function. I will also show that the inverse problem of determining the coefficients of the field equation in every cell at every interval of time, from the moments of the Green function, leads to nonuniqueness. For source points not on $\partial\mathcal{A}$, the moment equations are correct for either the infinite domain or the domains bounded by a perfectly absorbing and/or perfectly reflecting boundaries. For source points on $\partial\mathcal{A}$, corrections are necessary.

I consider a linear differential operator

$$\mathcal{L} = b_0(\mathbf{r},t) + \mathbf{b}_1(\mathbf{r},t)\cdot\nabla + b_2(\mathbf{r},t)\,\Delta$$

where the coefficients

$$\text{(C1)}\quad \begin{aligned} b_0(\mathbf{r},t) &= m(\mathbf{r},t) + \nabla\cdot\mathbf{v}(\mathbf{r},t)\\ \mathbf{b}_1(\mathbf{r},t) &= \mathbf{v}(\mathbf{r},t) - \nabla\, k(\mathbf{r},t)\\ b_2(\mathbf{r},t) &= -k(\mathbf{r},t)\,. \end{aligned}$$

The derivatives $\nabla\cdot\mathbf{v}(\mathbf{r},t)$ and $\Delta k(\mathbf{r},t)$ are assumed to exist. The effort-independent Green function $g(\mathbf{r},t|\mathbf{r}',t')$ satisfies the field equation

$$\text{(C2)}\quad \left[\frac{\partial}{\partial t} + \mathcal{L}\right] g(\mathbf{r},t|\mathbf{r}',t') = \delta(\mathbf{r}-\mathbf{r}')\delta(t-t')$$

in the space domain $\mathbf{r}\in\mathcal{A}$, subject to the causal condition

$$g(\mathbf{r},t|\mathbf{r}',t') = 0 \text{ if } t < t'\,.$$

The boundary of the domain $\mathcal{A}$ is $\partial\mathcal{A} = \partial\mathcal{A}_A + \partial\mathcal{A}_R$. In terms of the measuring space–time point $(\mathbf{r},t)$, the boundary conditions satisfied by $g(\mathbf{r},t|\mathbf{r}',t')$ are (Appendix A)

$$g(\mathbf{r},t|\mathbf{r}',t') = 0$$

in the spatial domain $\mathbf{r}\in\partial\mathcal{A}_A$ and

$$\mathbf{n}_\perp\cdot\nabla\, g(\mathbf{r},t|\mathbf{r}',t') = 0$$

in the spatial domain $\mathbf{r}\in\partial\mathcal{A}_R$ and where $\mathbf{n}_\perp$ is the unit vector normal to $\partial\mathcal{A}$ pointing outward.

To compute the even moments of $g$, multiply (C2) by the scalar $r^{2N}$ and integrate over domain $\mathcal{A}$ in the interval of time $\mathcal{T}_{-\infty} = [-\infty,t]$. Explicit integration with respect to time can only be performed on the term that contains the time derivative of $g$:

$$\int_{\mathcal{T}_{-\infty}} d\tau \int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N}\,\frac{\partial g(\mathbf{r},\tau|\mathbf{r}',t')}{\partial\tau} = \int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N}\, g(\mathbf{r},t|\mathbf{r}',t')$$

where I am allowed to change the order of integration by Fubinis' Theorem because the integrand is absolutely integrable (Royden 1968). I then have

$$\int_{\mathcal{T}_{-\infty}} d\tau \int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N}\, \mathcal{L}g(\mathbf{r},\tau|\mathbf{r}',t') = r'^{2N} - \langle r^{2N}\rangle(t|\mathbf{r}',t')$$

where

$$\langle r^{2N}\rangle(t|\mathbf{r}',t') = \int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N} g(\mathbf{r},t|\mathbf{r}',t')\,.$$

For the spatial integration in the l.h.s., I apply partial integration and the Divergence Theorem to those terms containing the gradient and the Laplacian of $g$. For the term containing the gradient of $g$, this results in

$$\int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N}\mathbf{b}_1(\mathbf{r},t)\cdot\nabla\, g(\mathbf{r},t\,|\,\mathbf{r}',t') = \oint_{\partial\mathcal{A}} dl\, r^{2N}\,\mathbf{n}_\perp\cdot\mathbf{b}_1(\mathbf{r},t)\, g(\mathbf{r},t\,|\,\mathbf{r}',t') - \int_{\mathcal{A}} d^2\mathbf{r}\,\nabla\cdot[r^{2N}\mathbf{b}_1(\mathbf{r},t)]\, g(\mathbf{r},t\,|\,\mathbf{r}',t')$$

while for the term with the Laplacian of $g$, the same procedure generates

$$\int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N} b_2(\mathbf{r},t)\,\Delta\, g(\mathbf{r},t|\mathbf{r}',t') = \oint_{\partial\mathcal{A}} dl\, r^{2N}\, b_2(\mathbf{r},t)\mathbf{n}_\perp\cdot\nabla g(\mathbf{r},t|\mathbf{r}',t') - \oint_{\partial\mathcal{A}} dl\,\mathbf{n}_\perp\cdot\nabla\,[r^{2N}\, b_2(\mathbf{r},t)]\, g(\mathbf{r},t|\mathbf{r}',t') + \int_{\mathcal{A}} d^2\mathbf{r}\,\Delta\,[r^{2N}\, b_2(\mathbf{r},t)]\, g(\mathbf{r},t|\mathbf{r}',t')\,.$$

The boundary integrals are used to satisfy the boundary conditions. After doing so, I am left with

$$\int_{\mathcal{T}_{-\infty}} d\tau \int_{\mathcal{A}} d^2\mathbf{r}\, E(\mathbf{r},\tau)\, g(\mathbf{r},\tau|\mathbf{r}',t') + \int_{\mathcal{T}_{-\infty}} d\tau\, C_E(\tau|\mathbf{r}',t') = r'^{2N} - \langle r^{2N}\rangle(t|\mathbf{r}',t')$$

where

$$E(\mathbf{r},\tau) = r^{2N} b_0(\mathbf{r},\tau) - \nabla \cdot [r^{2N} \mathbf{b}_1(\mathbf{r},\tau)] + \triangle [r^2 b_2(\mathbf{r},\tau)]$$

and

$$C_E(\tau|\mathbf{r}',t') = \begin{cases} -\oint_{\partial\mathcal{A}} dl\, r^{2N} k(\mathbf{r},t)\, \mathbf{n}_\perp \cdot \nabla\, g(\mathbf{r},\tau|\mathbf{r}',t') & \text{for } \partial\mathcal{A}_A \\ +\oint_{\partial\mathcal{A}} dl\, \mathbf{n}_\perp \cdot (\nabla r^{2N})\, k(\mathbf{r},t) g(\mathbf{r},\tau|\mathbf{r}',t') & \text{for } \partial\mathcal{A}_R\,. \end{cases}$$

Discretizing time in these expressions for a single interval of time for a source time $t_j$, I have

$$\left[\int_{\mathcal{A}} d^2\mathbf{r}\, E(\mathbf{r},t_j) g(\mathbf{r},t_j|\mathbf{r}',t_j) + C_E(t_j|\mathbf{r}',t_j)\right] = \frac{1}{\mathcal{T}_j}\left[r'^{2N} - \langle r^{2N}\rangle(t_{j+1}|\mathbf{r}',t_j)\right]$$

where

$$\langle r^{2N}\rangle(t_{j+1}|\mathbf{r}',t_j) = \int_{\mathcal{A}} d^2\mathbf{r}\, r^{2N} g(\mathbf{r},t_{j+1}|\mathbf{r}',t_j)\,.$$

As can be appreciated, the integrals on the l.h.s. of the expression have $g(\mathbf{r},t_j|\mathbf{r}',t_j)$ in the integrand. Making use of the initial property of $g$:

$$g(\mathbf{r},t_j|\mathbf{r}',t_j) = \delta(\mathbf{r}-\mathbf{r}')\,.$$

Integrating with the reminder that $\partial\mathcal{A} \subset \mathcal{A}$, I can conclude

$$E(\mathbf{r}',t_j) = r'^{2N} b_0(\mathbf{r}',t_j) - \nabla' \cdot \left[r'^{2N} \mathbf{b}_1(\mathbf{r}',t_j)\right] + \nabla'\left[r'^{2N} \mathbf{b}_2(\mathbf{r}',t_j)\right]$$

by use of the properties of the gradient of Dirac delta functions (Lighthill 1964; Butkov 1968) and the assumption that $\partial\mathcal{A}$ is piecewise smooth:

$$C_E(\mathbf{r}',t_j) = C_E(t_j|\mathbf{r}',t_j) = \begin{cases} 0 & \text{if } \mathbf{r}' \notin \partial\mathcal{A} \\ \nabla' \cdot [\boldsymbol{n}_\perp \mathbf{r}'^{2N} k(\mathbf{r}',t_j)] & \text{if } \mathbf{r}' \in \partial\mathcal{A}_A \\ \boldsymbol{n}_\perp \cdot (\nabla' r'^{2N}) k(\mathbf{r}',t_j) & \text{if } \mathbf{r}' \in \partial\mathcal{A}_R\,. \end{cases}$$

Evidently, $E(\mathbf{r}',t_j)$ is correct for points in $\mathcal{A}$ regardless of the boundaries. Therefore, it is also correct for the boundless domain. Henceforth, I shall ignore the terms with the source at the boundary of the domain because data are seldomly collected there.

Using the identities

$$\nabla' \cdot \mathbf{r}' = 2\,,$$

which is a consequence of $\mathcal{A} \in \mathbf{R}^2$,

$$\nabla' \mathbf{r}'^{2N} = 2N r'^{2N-2} \mathbf{r}'\,,$$

and

$$\nabla' \cdot (r'^{2N-2} \mathbf{r}') = 2N r'^{N-2}$$

to expand $E(\mathbf{r}',t_j)$ leads to the expression

$$E(\mathbf{r}',t_j) = r'^{2N} b_0(\mathbf{r}',t_j) - 2N r'^{2N-2}[\mathbf{r}' \cdot \mathbf{b}_1(\mathbf{r}',t_j)] - r'^{2N} \nabla' \cdot \mathbf{b}_1(\mathbf{r}',t_j) + 4N^2 r'^{2N-2} b_2[\mathbf{r}',t_j)] + 4Nr'^{2N-2}[\mathbf{r}' \cdot \nabla' b_2(\mathbf{r}',t_j)] + r_k'^{2N} \triangle' b_2(\mathbf{r}',t_j)\,.$$

Discretizing space with $\mathbf{r}_k$ as the source point and $\mathbf{r}_i$ as the receiver point, and substituting for $\{b_0,\mathbf{b}_1,b_2\}$ their expressions in terms of $\{m,\mathbf{v},k\}$, yields

$$E(\mathbf{r}_k,t_j) = r_k^{2N} m(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}[\mathbf{r}_k \cdot \mathbf{v}(\mathbf{r}_k,t_j)] - 4N^2 r_k^{2N-2} k(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}[\mathbf{r}_k \cdot \nabla_k k(\mathbf{r}_i,t_j)]$$

where I am using the notation

$$\nabla_i k(\mathbf{r}_i,t_j) = [\nabla k(\mathbf{r},t_j)]_{\mathbf{r}=\mathbf{r}_i}\,.$$

The scalar equations for the even moments of the Green function for source points $\mathbf{r}_k \notin \partial\mathcal{A}$ are then

$$E(\mathbf{r}_k,t_j) = \frac{1}{\mathcal{T}_j}[r_k^{2N} - \langle r_i^{2N}\rangle(t_{j+1}|\mathbf{r}_k,t_j)]$$

and where

$$\langle r_i^{2N}\rangle(t_{j+1}|\mathbf{r}_k,t_j)] = \sum_{i=1}^{n} \mathcal{A}_i r_i^{2N} g(\mathbf{r}_i,t_{j+1}|\,\mathbf{r}_k,t_j)\,.$$

With these, I can generate the zeroth ($N = 0$), second ($N = 1$), . . ., moments of the Green function.

To derive the odd-moments equation, I can initially multiply the field equation for the Green function by $r^{2N}\mathbf{r}$ and perform

analogous operations using the algebra and calculus of dyads. This leads to the vector $\boldsymbol{O}(\mathbf{r}_k,t_j)$ analogous to the scalar $E(\mathbf{r}_k,t_j)$ given by

$$\boldsymbol{O}(\mathbf{r}_k,t_j) = r_k^{2N}\mathbf{r}_k m(\mathbf{r}_k,t_j) - r_k^{2N}\mathbf{v}(\mathbf{r}_k,t_j) - 2Nr_k^{2N-2}\mathbf{r}_k[\mathbf{r}_k\cdot\mathbf{v}(\mathbf{r}_k,t_j)]$$
$$-4N(N+1)r_k^{2N-2}\mathbf{r}_k k(\mathbf{r}_k,t_j) - r_k^{2N}\nabla_k k(\mathbf{r}_k,t_j)$$
$$-2Nr_k^{2N-2}\mathbf{r}_k[\mathbf{r}_k\cdot\nabla_k k(\mathbf{r}_k,t_j)]$$

for $\mathbf{r}_k \notin \partial\mathcal{A}$. The vector equations for the odd moments of the Green function are then

$$\boldsymbol{O}(\mathbf{r}_k,t_j) = \frac{1}{\mathcal{T}_j}[r_k^{2N}\mathbf{r}_k - \langle r_i^{2N}\mathbf{r}_i\rangle(t_{j+1}|\mathbf{r}_k,t_j)]$$

where

$$\langle r_i^{2N}\mathbf{r}_i\rangle(t_{j+1}|\mathbf{r}_k,t_j) = \sum_{i=1}^{n}\mathcal{A}r_i^{2N}\mathbf{r}_i g(\mathbf{r}_i,t_{j+1}|\mathbf{r}_k,t_j)\,.$$

With these, I can generate the first ($N = 0$) , third ($N = 1$), . . ., moments of the Green function.

# REVIEWS/SYNTHÈSES

# Microalgal Pigment Assessments Using High-Performance Liquid Chromatography: A Synopsis of Organismal and Ecological Applications

David F. Millie[1]

*U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 80124, USA*
*and*
*Loyola University, Department of Biological Sciences, 6363 St. Charles Avenue, New Orleans, LA 70118, USA*

Hans W. Paerl

*University of North Carolina – Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, USA*

and James P. Hurley

*Wisconsin Department of Natural Resources, Bureau of Research, and University of Wisconsin, Water Chemistry Program, 660 North Park Street, Madison, WI 53706, USA*

Millie, D.F., H.W. Paerl, and J.P. Hurley. 1993. Microalgal pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications. Can. J. Fish. Aquat. Sci. 50: 2513–2527.

Past and current efforts at identifying microalgal phylogenetic groups rely largely on microscopic evaluation, which requires a high level of taxonomic skill, may take considerable time, can be variable among personnel, and does not allow characterization of the physiological status of the taxa. High-performance liquid chromatography (HPLC) has proven effective in rapidly separating and distinguishing chlorophylls, chlorophyll-degradation products, and carotenoids within monotypic and mixed algal samples. When coupled with absorbance and/or fluorescence spectroscopy, HPLC can accurately characterize phylogenetic groups and changes in community composition and yield information concerning microalgal physiological status, production, trophic interaction, and paleolimnology/paleooceanography. The recent widespread occurrence of toxic and noxious phytoplankton blooms has necessitated the use of remote imagery of pigment and reflectance "signatures" for monitoring and predicting bloom distribution. Because HPLC allows the processing of large numbers of samples from numerous locations relatively quickly, it is ideally suited for large-scale "ground truthing" of remotely sensed imagery. Coupled with rapidly evolving computer-based remote sensing technologies, HPLC-based pigment analyses may provide accurate assessments of aquatic biogeochemical flux, primary production, trophic state, water quality, and changes therein on local, regional, and global scales.

Les efforts antérieurs et actuels d'identification des groupes phylogénétiques de microalgues reposent en grande partie sur une évaluation microscopique, qui nécessite de grandes compétences en taxonomie, peut prendre beaucoup de temps, peut être variable d'une personne à l'autre, et ne permet pas une caractérisation de l'état physiologique des taxons. La chromatographie liquide à haute performance (CLHP) s'est révélée efficace pour séparer et distinguer rapidement les chlorophylles, les produits de décomposition de la chlorophylle et les caroténoïdes dans des échantillons d'algues monotypiques ou mélangées. Lorsqu'elle est combinée à la spectroscopie d'absorbance ou de fluorescence, la CLHP permet une caractérisation précise des groupes phylogénétiques et des modifications de la composition de la communauté et permet d'obtenir des informations sur l'état physiologique des microalgues, leur production, l'interaction trophique et la paléolimnologie et la paléoocéanographie. L'apparition récente à grande échelle d'efflorescences phytoplanctoniques toxiques et nuisibles a nécessité l'utilisation de l'imagerie de télémesure des pigments et des « signatures » de réflectance pour surveiller et prévoir la répartition des efflorescences. Parce que la CLHP permet le traitement assez rapide d'un très grand nombre d'échantillons provenant de nombreux endroits, cette technique est théoriquement bien adaptée pour la « vérification au sol » sur une grande échelle de l'imagerie obtenue par télémesure. Combinée à des techniques de télédétection assistées par ordinateur en évolution rapide, les analyses de pigment par CLHP peuvent fournir des évaluations précises du flux biogéochimique aquatique, de la production primaire, de l'état trophique, de la qualité de l'eau et de leurs modifications au niveau local, régional et mondial.

*Received January 22, 1993*
*Accepted June 1, 1993*
*(JB766)*

*Reçu le 22 janvier 1993*
*Accepté le 1 juin 1993*

[1]Author to whom correspondence should be addressed.

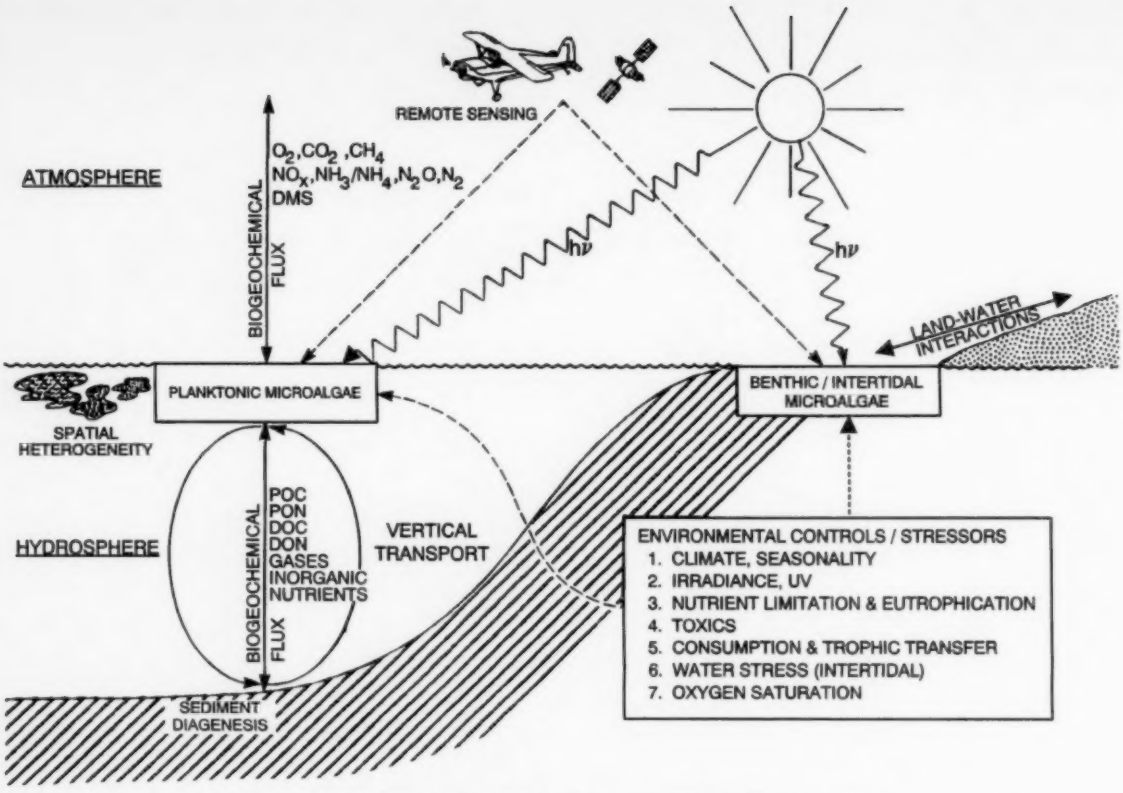



FIG. 1. Microalgal production in relation to energy and biogeochemical cycling.

Plant pigments capture solar energy, mediate its conversion to chemical energy, and ultimately control primary production and carbon and nutrient flux in the biosphere (Fig. 1). The major pigment groups, chlorophylls (Chls), carotenoids, and phycobilins, and individual pigments within these groups are diagnostic of specific plant taxa. Recently, it has been recognized that both the central biogeochemical roles and taxon-specific attributes of plants may be of use in rapidly and unequivocally identifying and quantifying microalgae, the dominant contributors to freshwater and marine primary production.

Fixed-wavelength spectrophotometric absorbance and fluorometric emission/excitation analyses have been regarded over the past two decades as standard techniques for quantifying microalgal Chls, most notably Chl *a*; these analyses are easily executed, applicable to a wide array of aquatic ecosystems, and capable of quantifying a pigment group universally present in microalgae (see Table 1). Although sample collection, processing, and analyses are reasonably similar among researchers, variation among these procedures often are necessary to appropriately characterize ecologically and physiologically diverse algal assemblages. As a result, traditional pigment extractions and analyses may lead to differing quantifications of phytoplankton biomass under contrasting environmental conditions.

A serious limitation of spectrophotometric and fluorometric techniques is their inability to analyze and quantify multipigment mixtures often encountered in natural samples. In mixed algal assemblages, as many as 20 diagnostic pigments may cooccur. High performance liquid chromatography (HPLC) has proven effective in rapidly separating photosynthetic pigments, prior to spectrophotometric and fluorometric detection. In this manner, microalgae known to contain unique pigments and combinations of pigments (such as diagnostic carotenoids, phycobilins, specific combinations of Chls *a*, *b*, and *c* and Chl derivatives; Table 2) may be detected and quantified. Recently, rapid (<0.5 s) multiwavelength scanning spectrophotometers and photodiode array spectrophotometers (PDAS) have become available as HPLC detectors and are quite useful for identification of individual pigments (see Fig. 2). As such, HPLC and spectroscopic analyses of multipigment natural assemblages open up new avenues for detecting, characterizing, and quantifying diverse microalgae within a single sample as quickly as 20 min.

A sufficient number of aquatic scientists have implemented HPLC–PDAS in their studies to warrant the evaluation of these technologies in terms of their application to basic and applied research (also see Gieskes 1991). In this review, we assess the utility of HPLC–PDAS within organismal and ecological analyses and discuss the potential applications of these analyses on local, regional, and global scales.

## Analytical Perspectives

Fixed-wavelength spectrophotometric absorbance analysis relies upon extraction of algal pigments into organic solvents, absorbance measurements of the extracted pigment solution at specific wavelengths, and the estimation of Chl pigments based

TABLE 1. Comparison of spectrophotometric, fluorometric, and high-performance liquid chromatographic (HPLC) methodologies for algal pigment analyses.

| | Spectrophotometric | Fluorometric | HPLC |
|---|---|---|---|
| Pigments detected | Chlorophylls, pheopigments, carotenoids, phycobilins | Chlorophylls, pheopigments, phycobilins | Chlorophylls, pheopigments carotenoids, phycobilins[a] |
| Detection limits | ~0.02 $mg \cdot m^{-3}$ | $ng \cdot m^{-3}$ | Dependent upon method and equipment; $pg \cdot m^{-3}$ |
| Interferences | Accessory and bacterial chlorophylls | Accessory chlorophylls | Chemical compounds derived from sample preparative materials |
| Advantages | Fast; easily executed | Fast; easily executed; in vivo measurements amenable to continuous in situ monitoring of cells | Individual pigments (including chlorophyll derivatives) detected and potentially quantified in 20–30 min; spectral "signatures" of extracted pigments can be constructed |
| Disadvantages | Overlapping spectra of pigments do not allow accurate quantification; wide variety of published pigment absorption coefficients and "standardized" equations produce variable results | Inability to resolve accessory chlorophylls and chlorophyll derivatives | Equipment needs; cost may be prohibitive to some researchers; accurate quantification dependent upon cross-calibration of pigment standards; availability of adequate pigment standards (see Analytical Evaluation section) |

[a]See Swanson and Glazer (1990); however, a suitable HPLC methodology for the separation and quantification of major phycobiliproteins from natural assemblages has not been developed.

on "standardized" equations using specific absorption coefficients (Richards and Thompson 1952; Parsons and Strickland 1963; Strickland and Parsons 1972). Fluorometric emission/excitation analysis relies upon the absorption of quanta (photons) by Chl *a*, the subsequent decrease in energy by Chl *a* from an excited to a ground state, and the resulting emission of radiation. Fluorometric analyses can be conducted upon both Chl *a* extracted into organic solvents (in vitro) and Chl *a* intact within cells (in vivo) (e.g., Yentsch and Menzel 1963; Holm-Hansen et al. 1965; Lorenzen 1966; Strickland 1968; Yentsch and Yentsch 1979). The fluorometric method often is preferred over the spectrophotometric method because it is more sensitive, faster, requires smaller sample volumes, not dependent upon precise wavelength alignment needed for the instrument (therefore, better suited for routine laboratory and field studies), and not critically dependent upon cuvette handling and matching (Smith et al. 1981).

However, technical and interpretational problems exist with both the spectrophotometric and fluorometric methods. Discrepancies arise with the spectrophotometric method due to the large variability among both published adsorption coefficients used within "standardized" equations and the equations themselves. The inability to resolve Chls *b* and *c*, Chl derivatives (pheophytins, pheophorbides, chlorophyllides, red-shifted Chl *a*) and carotenoids and the variability among in vivo fluorescence yields of Photosystem II (due to differences in light fluoresced from different taxa, a taxon's physiological state, and the taxon's physiological history of light exposure) are limitations of the fluorometric method (Yentsch and Yentsch 1979; Smith et al. 1981; Gieskes and Kraay 1982). Both methodologies rely upon obtaining significant and detectable "signals" from in vitro or in vivo pigments. In instances where phytoplankton biomass is small, such as in ultraoligrotrophic Crater Lake, Oregon (USA), the sensitivity of these methods may not allow for accurate pigment quantification (J. Salinas, Cascade Research Group, Murphy, OR 97553, USA personal communication). Additionally, researchers addressing spectrophotometric and fluorometric methodology in both freshwater (Jacobsen and Rai 1990) and marine (Trees et al. 1985) systems have reported that these methods may not accurately quantify Chl *a* concentrations in water samples. The fluorometric method over- and underestimates Chl *a* and Chl-degradation products, respectively, when large amounts of Chl *c* are present within samples, which is often the case when bacillariophytes, chrysophytes, and dinophytes are dominant. The reverse occurs when Chl *b* is present (Gieskes and Kraay 1982, 1984; Trees et al. 1985).

Chromatographic separation and characterization of individual Chls, Chl derivatives, and carotenoids provide researchers with definitive information for characterization of algal assemblages. For a chromatographic technique to be useful, it must be rapid, simple to execute, resolve pigments to extremely low levels of detection, be innocuous, and cause no chemical change to the pigments (Jeffrey 1981). Using paper and thin-layer chromatography, Jeffrey (1961, 1965, 1968, 1974, 1981) effectively separated and characterized lipophilic pigments from natural and laboratory-culture phytoplankton assemblages. However, although these chromatographic methodologies are ideal for separating Chls, Chl derivatives, and carotenoids, the quantitative estimations of individual pigments are tedious. After successful separation on paper on thin-layer cellulose surfaces, individual pigments must be removed from the chromatographic surface and "eluted" into an organic solvent. Individual pigment concentrations then are determined spectrophotometrically using appropriate extinction coefficients (e.g., Jeffrey 1968).

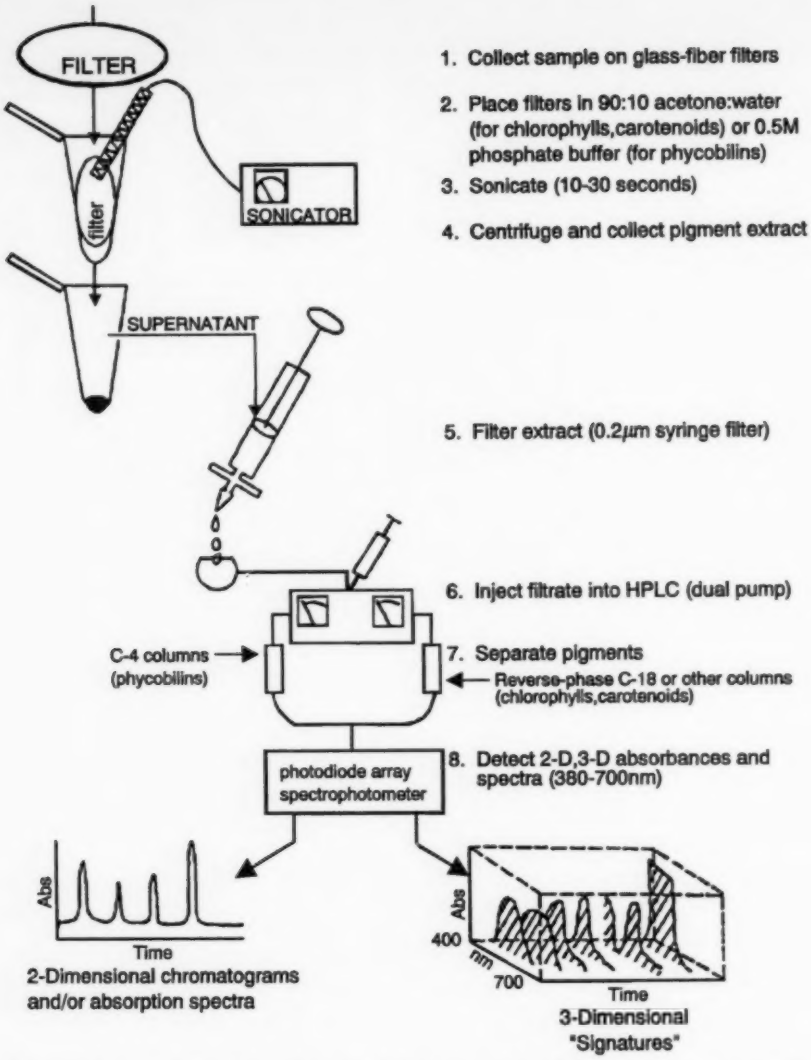



FIG. 2. Preparative and analytical procedures for high-performance liquid chromatographic – photodiode array spectrophotometry of microalgal pigments.

Coupled with absorbance and/or fluorescence spectroscopy (Fig. 2), HPLC can accurately separate and quantify pigments at extremely low detection levels within monotypic and mixed algal samples (see Brown et al. 1981; Mantoura and Llewellyn 1983; Paerl et al. 1984; Wright and Shearer 1984; Bidigare et al. 1985; Wright et al. 1991; Kraay et al. 1992; Van Heukelem et al. 1992). As such, these technologies provide a means for facilitating rapid characterization of pigments diagnostic for phylogenetic groups (Table 2) and for monitoring changes in community composition. Additionally, the characterization of diagnostic microalgal pigments via PDAS, coupled to rapidly evolving multichannel and scanning remote sensing technologies, provides promise that quantitative and qualitative assessments of aquatic biogeochemical flux, primary production, trophic state, water-quality conditions, and changes therein can be accomplished (see Fig. 1).

## Phylogenetic and Community Characterization

Identification and characterization of microalgal phylogenetic groups are based on the presence/absence of diagnostic pigments (Table 2) and rely largely upon microscopic evaluation. However, accurate microscopic evaluation requires a level of taxonomic skill and training often not available to the researcher, may take considerable time, can be highly variable among personnel, and does not allow characterization of the physiological status of the taxa (see below). HPLC-based pigment analyses can help circumvent the analytical and interpretational limitations in the microscopic detection of indicator algae, particularly those of small and/or fragile cells.

Diagnostic Chl, carotenoid, and phycobilin pigments, either singly or in combination, have been used for chemosystematic identification of phytoplankton assemblages (Table 2). Additionally, the presence of the lipid-soluble UV-absorbing

TABLE 2. Phytoplankton pigments useful as diagnostic phylogenetic/physiological markers. A phylogenetic group is designated if any or all of its taxa are reported to possess the pigment. Phycobilins include isomers diagnostic at the genera/species level.

| Pigment | Phylogenetic groups | Reference(s) |
|---|---|---|
| Chlorophylls | | |
| *a* | In all groups, sole chlorophyll in cyanophytes and eustigmatophytes | Jeffrey 1980; Rowan 1989 |
| *b* | Chlorophytes, euglenophytes, prasinophytes, prochlorophytes | Paerl et al. 1984; Rowan 1989; Guillard et al. 1991 |
| $c_1$ | Bacillariophytes, chrysophytes, prymnesiophytes, raphidophytes, xanthophytes | Jeffrey et al. 1975; Jeffrey 1976, 1989; Stauber and Jeffrey 1988 |
| $c_2$ | Bacillariophytes, cryptophytes, dinophytes prymnesiophytes, raphidophytes, xanthophytes | Jeffrey et al. 1975; Jeffrey 1976, 1989 |
| $c_3$ | Bacillariophytes, chrysophytes, dinophytes, prymnesiophytes | Stauber and Jeffrey 1988; Bidigare 1989; Jeffrey 1989; Bidigare et al. 1990a; Wright et al. 1991 |
| 8-desethyl, 8-vinyl *a* | Prochlorophytes | Goericke and Repeta 1992 |
| 8-desethyl, 8-vinyl *b* | Prochlorophytes | Goericke and Repeta 1992 |
| Magnesium 2,4-divinylpheoporphyrin $a_5$ monomethyl ester | Prasinophytes | Ricketts 1966, 1970; Hooks et al. 1988 |
| Carotenoids | | |
| Alloxanthin | Cryptophytes | Chapman 1966; Gieskes and Kraay 1983a |
| 19′-Butanoyloxyfucoxanthin | Dinophytes, prymnesiophytes, raphidophytes | Wright and Jeffrey 1987; Bjørnland et al. 1988; Bjørnland 1989 |
| Crocoxanthin | Cryptophytes | Chapman 1966; Wright et al. 1991 |
| Dinoxanthin | Dinophytes, prymnesiophytes | Jeffrey 1961, 1968 |
| Echinenone | Cyanophytes, prochlorophytes | Hertzberg and Liaaen-Jensen 1966a, 1966b; Paerl et al 1984; Foss et al. 1987 |
| Fucoxanthin | Bacillariophytes, chrysophytes, dinophytes, pyrmnesiophytes | Jeffrey 1961; Wright and Jeffrey 1987; Bjørnland et al. 1988 |
| 19′-Hexanolyloxyfucoxanthin | Dinophytes, pyrmnesiophytes | Arpin et al. 1976; Tangen and Bjørnland 1981; Gieskes and Kraay 1986b; Wright and Jeffrey 1987; Bjørnland and Liaaen-Jensen 1989 |
| Lutein | Chlorophytes, prasinophytes | Jeffrey 1961, 1968; Ricketts 1970; Wright et al. 1991 |
| Monadoxanthin | Cryptophytes | Chapman 1966; Wright et al. 1991 |
| Myxoxanthophyll | Cyanophytes | Hertzberg and Liaaen-Jensen 1966a, 1966b, 1969a, 1969b; Hertzberg et al. 1971 |
| Oscillaxanthin | Cyanophytes | Hertzberg and Liaaen-Jensen 1969b; Hertzberg et al. 1971 |
| Peridinin | Dinophytes | Jeffrey 1961, 1968; Strain et al. 1971; Jeffrey et al. 1975 |
| Prasinoxanthin | Prasinophytes | Foss et al. 1984; Guillard et al. 1991 |
| Pyrrhoxanthin | Dinophytes | Aakermann and Liaaen-Jensen 1992 |
| Siphonaxanthin | Chlorophytes, euglenophytes, prasinophytes | Jeffrey 1968; Rowan 1989 |
| Vaucheriaxanthin | Eustigmatophytes, xanthophytes | Stansky and Hager 1970; Bjørnland and Liaaen-Jensen 1989 |
| Zeaxanthin | Cryptophytes, cyanophytes prasinophytes, prochlorophytes | Chapman 1966; Hertzberg and Liaaen-Jensen 1966a, 1966b; Hertzberg et al. 1971; Guillard et al. 1985 |
| Phycobilins | | |
| Allophycocyanin | Cyanophytes | O'Carra and O'h Eocha 1976; Rowan 1989 |
| Phycocyanin | Cyanophytes, cryptophytes | O'Carra and O'h Eocha 1976; Rowan 1989 |
| Phycoerythrin | Cyanophytes, cryptophytes, rhodophytes | O'Carra and O'h Eocha 1976; Rowan 1989 |

sheath pigment, scytonemin (Garcia-Pichel and Castenholz 1991), in microbial mats is indicative of sheathed cyanophytes, such as *Lyngbya* spp., *Calothrix* spp. and *Scytonema* spp., which form the fabric of the mat. Although pigments may vary among cells within a taxon or between taxa, the abundances of these diagnostic pigments generally reflect the major distributions of the respective phytoplankton groups. Barlow et al. (1993), however, suggested that the use of certain diagnostic pigments (such as 19′-butanoyloxyfucoxanthin) should be exercised with caution due to their presence in more than one algal group. Several researchers (Gieskes and Kraay 1983b; Bidigare et al. 1986; Gieskes et al. 1988; Everitt et al. 1990; Barlow et al. 1993) have used diagnostic pigment ratios to quantitatively assess the temporal and spatial dynamics of biomass and community structure.

Identification of algal phylogenetic groups through pigment

"signatures" presently is limited to the division or class level. More precise phylogenetic classifications should be made with caution. For example, the presence/absence of select carotenoids has been included for class or order distinctions within some chlorophytes (Fawley and Lee 1990; Guillard et al. 1991). Analyses have indicated that the distributions of several of these carotenoids (loroxanthin, siphonaxanthin, siphonein) are disjunct and do not have an apparent systematic utility (Fawley 1991). Prasinoxanthin has been used as a chemosystematic indicator to include (several strains of) the coccoid ultraplankter *Pycnococcus provasolii* Guillard in the micromonadophyte order Mamiellales (Guillard et al. 1991). However, because considerable variation in the pigment composition of organisms that contain both prasinoxanthin and magnesium 2,4-divinyl phaeoporphyrin $a_5$ monomethyl ester exist, it should not be assumed that prasinoxanthin always indicates the presence of members of Mamiellales. As such, the taxonomic placement of an organism should not be solely based upon the presence/absence of an individual pigment, but rather on a combination of relevant characters (Fawley 1992).

If chloroplast-bound pigments are to be used to characterize algae, investigators must consider the actual and potential occurrence of endosymbiosis. According to the endosymbiotic theory, chloroplasts and mitochondria arose from free-living prokaryotes which entered eucaryotic cells. If this endosymbiosis occurred recently (on a evolutionary time scale), diagnostic pigments may not always be reliable indicators of a taxon's phylogeny (Bjørnland 1989). For example, among most autotrophic dinophytes, peridinin is the primary light-harvesting pigment and is considered the diagnostic carotenoid for the group. However, several ecologically and economically important dinophytes (including the Florida "red tide" *Gymnodinium breve* Davis) do not contain peridinin but rather have fucoxanthin, 19′-hexanoyloxyfucoxanthin, and/or 19′-butanoyloxyfucoxanthin, either singly or in combination, as the primary light-harvesting pigment (Jeffrey et al. 1975; Tangen and Bjørnland 1981; Bjørnland et al. 1988; Hooks et al. 1988; Bjørnland 1989; Millie et al. 1993). These 19′-acylofucoxanthins also are found in several prymnesiophytes and chrysophytes (Bjørnland and Liaaen-Jensen 1989). Therefore, if the chloroplasts of the fucoxanthin-based dinophytes have been acquired through endosymbiosis, the donors might be sought among these classes (Bjørnland et al. 1988). Reports of other dinophytes containing vestigial bacillariophyte-, chrysophyte-, cryptomonad-, and cryptophyte-like endosymbionts (Tomas and Cox 1973; Jeffrey and Vesk 1976; Withers et al. 1977; Watanabe et al. 1990) support this theory.

Due to the variable distribution of pigments among algal groups, caution should be exercised when choosing diagnostic pigments as indicators of biomass. Chl *a* often is used as a biomass indicator in aquatic systems. However, Chl *a* content per cell differs among taxa and the alteration of Chl *a* content by a taxon in response to environmental conditions has largely been ignored in biomass estimations. Additionally, because Chl extraction is variable depending upon the chosen solvents and algal assemblage sampled (Bowles et al. 1985; Lloyd and Tucker 1988), Chl-*a* based biomass estimates always may not quantify algal groups equally. Fluorometric, spectrophotometric, and many HPLC methodologies cannot differentiate between Chl *a* and 8-desthyl, 8-vinyl-Chl *a*, the major photosynthetic pigment in the free-living prochlorophyte *Prochlorococcus marinus* Chisholm. Because this organism may dominate samples from the Chl maximum and euphotic zone in the Sargasso Sea, inaccurate quantification of Chl *a* in such samples may result (Goericke and Repeta 1992).

In contrast, several carotenoids have proven to be less variant than Chl *a* to environmental conditions and, depending upon the type of information desired, may be better indicators of algal biomass. Peridinin was highly correlated with dinophyte abundance in a northern Wisconsin (USA) bog and proved to be an accurate indicator of temporal periodicity and diurnal-migration patterns (B. Sanderson, Center For Limnology, University of Wisconsin, Madison, WI 53706, USA, unpublished data). Fucoxanthin was more indicative of bacillariophyte growth rate than Chl *a* in seawater dilution experiments assessing phytoplankton growth within the subarctic Pacific Ocean (Strom and Welschmeyer 1991). However, xanthophyll to Chl *a* ratios vary dramatically within and among phylogenetic groups and are dependent upon algal growth stage and certain environmental parameters (Buma et al. 1991; Wilhelm and Manns 1991). Therefore, methods that utilize such parameters may not accurately estimate phytoplankton biomass. Wilhelm et al. (1991) noted that although HPLC pigment analyses and microscopic enumeration techniques provided similar population dynamics, absolute values of the relative contribution of algal groups differed substantially. This difference was attributed to different reference parameters among the methods; pigment analyses provided the relative contribution per unit Chl *a* whereas cell enumeration was based upon biovolume which could not differentiate between active and inactive cells. Obviously, pigment analyses coupled with microscopic enumeration would provide the most accurate characterization of algal assemblages (also see Soma et al. 1993).

Bacteriochlorophyll (BChl) analyses by HPLC have been used to characterize photosynthetic bacterial composition in both freshwater (Hurley and Watras 1991) and marine (Repeta et al. 1989) systems. Hurley and Watras (1991) characterized previously unidentified layers of phototrophic bacteria in two Wisconsin (USA) lakes. In one lake, a bacterial layer containing BChl *d* previously had been misidentified by traditional Chl analyses as a deep layer of pheopigment. In other Wisconsin lakes, dense layers of phototrophic bacteria in late summer contained BChls *a*, *d*, and *e*, (indicative of purple, green, and brown sulfur bacteria, respectively) in concentrations routinely in excess of 100 $\mu g \cdot L^{-1}$ (Hurley and Garrison 1993). Because BChls absorb at or near wavelengths used in the "standardized" equations of traditional spectrophotometric methods (approximately 665 nm), these protocols would have misidentified the BChls as algal-derived Chls and may have led to misinterpretations of planktonic distributions and processes (also see Curaco and Puccoon 1986; Hurley and Watras 1991). Similar analyses could be logically extended to marine and estuarine microbial mats where the relationship between algae and photosynthetic bacteria is well documented (Paerl et al. 1989a, 1989b; Palmisano et al. 1989).

## "Biomarkers" for Growth, Physiology, and Production

Values of carbon (C)-specific biomass and growth rates often are used for comparing spatial and temporal dynamics of phytoplankton. However, it is difficult to separate the phytoplankton component from other particulate organic matter in whole-water samples and when accomplished, values vary depending upon the taxa and their physiological state (Redalje

1993). Redalje and Laws (1981) and Redalje (1983) developed techniques for accurately estimating community growth rates and biomass using $^{14}C$ labeling of Chl *a* and particulate matter and the subsequent separation of these components by thin-layer chromatography. After modifying this methodology for HPLC, Gieskes and Kraay (1989) estimated the contribution of bacillariophytes, cyanophytes, and prymnesiophytes to the total phytoplankton production in the Banda and Arafura seas through $^{14}C$ labeling of Chl *a* and taxon-specific carotenoids. More recently, $^{14}C$ labeling of pigments has been extended to estimate turnover rates for the Chl *a* tetrapyrole ring and its phytol side chain and the biosynthesis and turnover rates for Chl *a* and specific carotenoids (Goericke and Welschmeyer 1992a, 1992b, 1993a, 1993b). However, because some pigments display large values for turnover rates and phytoplankton growth can be severely unbalanced during a natural photocycle or during photoacclimation, $^{14}C$ labeling of algal pigments may produce estimates with significant sources of error, thereby leading to invalid production rates for certain phylogenetic groups (Gieskes 1991; Goericke and Welschmeyer 1993a, 1993b). Additionally, impure radiolabeled pigment preparations can lead to overestimation of pigment-specific activities and may bias estimates of phytoplankton biomass and growth rate (Goericke 1992; also see Jespersen et al. 1992).

Radioactive labeling and subsequent "tracing" of algal pigments and pigment precursors open up exciting new avenues for ecological and physiological research. Such techniques will allow investigators to quantify, and potentially model, pigment and pigment-precursor turnover, conversion (i.e., xanthophyll "cycling," see Stansky and Hager 1970; Bidigare 1989; Kohata and Watanabe 1989), and degradation rates in response to environmental and endogenous factors known to affect pigment regulation within microalgae. Additionally, stable isotopic analyses of HPLC-separated and purified pigments may prove useful in delineating and characterizing sources of nutrients (atmospheric, terrestrial, autochthonous regenerated) that control microalgal production and bloom dynamics. In particular, stable nitrogen (N) isotopes having unique $\delta^{15}N$ values can be "traced" from their sources into algal biomass and potentially Chl *a* synthesis (Paerl et al. 1993a, 1993b). In light of the role that "new" N inputs play in coastal and estuarine primary production and eutrophication dynamics (Eppley and Peterson 1979; Legendre and Gosselin 1989; Paerl 1993), the combination of HPLC-based pigment analyses with mass-spectrometry-based N isotope characterization techniques may identify "new" N sources and their biogeochemical and trophic fates in the marine environment.

Because the photosynthetic apparatus reflects the response of algae to changing environmental and endogenous variables, alterations in pigment "signatures" developed through HPLC provide a means to monitor the physiological state of an algal assemblage. For example, carotenoid accumulation is considered an adaptive response by algae for protection against photooxidation (Guillard et al. 1985; Gieskes and Kraay 1986a, 1986b; Bidigare et al. 1989b, 1993; Johnsen and Sakshaug 1993) and may dramatically affect primary production potential (Paerl et al. 1983, 1985; Paerl 1984; Millie et al. 1990a, 1990b). Photoprotection may involve both active and passive mechanisms; primary carotenoids quench photosensitizing pigments and free radicals whereas secondary carotenoids absorb photons before they reach the photosynthetic mechanism (Bidigare et al. 1993). Kohata and Watanabe (1989) determined diel changes in lipophilic pigments, C, and N in a prasinophyte and noted that Chls and most carotenoids increased during the light period due to photosynthesis but decreased during the dark period due to phased cell division. Additionally, documentation of alterations in diagnostic carotenoid pigments may provide the basis upon which to discern nutrient depletion and photodegradation/oxidation processes in phytopolankton (Millie et al. 1992b; Kobayashi et al. 1993; Geider et al. 1993).

The presence of Chl degradation products (chlorophyllides, pheophorbides, pheophytins, pyropheophytins appear to be a reliable indicator of physiological stress in both natural and laboratory-culture phytoplankton assemblages. For example, Jeffrey and Hallegraeff (1987) reported that senescent cells with high chlorophyllase activity produced increased levels of chlorophyllide *a*. Gowen et al. (1983) suggested that chlorophyllide *a* increases reflected phytoplankton physiological state and/or the nutrient status of the water column within two Scottish lochs. In support of this hypothesis, Ridout and Morris (1985) observed that increases in chlorophyllide *a* concentrations coincided with maximum nutrient limitation in experimental bags within Loch Ewe (Scotland). However, chlorophyllase activity within cells can be activated by harvesting techniques (filtration and centrifugation) prior to pigment extraction and activity appears to differ among taxa. As such, the presence of chlorphyllide *a* on chromatograms must be considered a possible analytical artifact (Jeffrey and Hallegraeff 1987). Schoch et al. (1981) reported that *Euglena* spp. produced steadily increasing amounts of pyropheophytin relative to pheophytin under prolonged periods of darkness and suggested that pyropheophytin was an intermediate in Chl degradation.

HPLC-based pigment analyses may assist in identifying a "biomarker" for toxic and taste/odor metabolites. In many microalgae, the synthesis of these compounds appear to be closely linked with and/or regulated by environmental or endogenous factors affecting pigment biosynthesis, physiological state, and growth stage (Naes et al. 1989; Utkilen and Gjølme 1992; Bafford et al. 1993). Such a rapid, objective screening and "biomarker" protocol could be readily deployed, is economically feasible, and is well suited to water-quality monitoring over time and space. Additionally, some microalgae, such as the halotolerant chlorophyte *Dunaliella bardawil* Ben-Amotz & Avron, have been exploited as commercial sources of β-carotene (e.g., Ben-Amotz and Avron 1980; Shaish et al. 1991). HPLC can assist in rapidly screening for taxa having optimal carotene production for a variety of commercial applications.

## Trophic Fate Applications

The taxonomic composition and distribution of phytoplankton assemblages are created and subsequently maintained or altered by an interacting set of physical and biological processes. HPLC–PDAS can serve as a valuable tool for accurate characterization of trophic processes (planktonic grazing, predator–prey relationships) that affect phytoplankton assemblages (Kleppel and Pieper 1984; Welschmeyer and Lorenzen 1985; Bidigare et al. 1986; Gieskes and Kraay 1986b; Klein and Sournia 1987; Kleppel et al. 1988, 1991). Because of the liability of many pigment molecules and the heterotrophic metabolic changes pigments may undergo (e.g., Nelson 1989, 1993; Bianchi and Findley 1991; Bianchi et al. 1991), studies concerning trophic processes must focus upon pigment dynamics occurring within a few trophic "steps" from the parent algal

pigment. For example, periods of intense grazing pressure can be identified through fluxes in pheophorbide (a processing indicator) and selected carotenoids within pigment "signatures" from whole-water and sediment trap samples (see Carpenter et al. 1986).

Gut pigment compositions of mesozooplankton can be used to infer the taxonomic composition of the diet. Such analyses are faster, more precise, more quantifiable, and have a greater detection for algal taxa than standard microscopic gut analyses. Estimates of pigment-specific ingestion rates also may be inferred when gut pigment analyses are coupled with measurements of pigment passage rates through the gut. However, algal pigment destruction during gut passage appears to differ amongst zooplankton, and further development of this approach requires a comprehensive understanding of pigment conservation and destruction (see Klein et al. 1986; Gieskes et al. 1991). For example, in laboratory studies in which cultured algae were fed to a copepod, the carotenoids fucoxanthin, zeaxanthin, and lutein (indicative of bacillariophytes, cyanobacteria, and chlorophytes, respectively) were conserved (85–100% recovery). The carotenoid peridinin (indicative of dinophytes) was not completely conserved (60% recovery), but the loss was relatively consistent (G. Kleppel, Oceanographic Center, Nova University, Dania, Fl 33004, USA, unpublished data). In contrast, Head and Harris (1992) reported that 100% of the fuxoanthin, 9–82% of the Chl *a*, and 70–100% of the Chl *c* were destructed within guts of *Calanus* sp. grazing on bacillariophytes. Radioisotope labeling of diagnostic pigments may assist in accurately quantifying and depicting degradation rates of individual pigments.

Studies concerning trophic pigment dynamics also must consider microalgal pigment composition and destruction within guts of microzooplankton. Microzooplankton are abundant in aquatic systems and of tremendous trophic importance. Although these organisms may have the same grazing impact as the mesozooplankton, they largely exploit the nanoplankton that are unavailable to the larger zooplankters. Microzooplanton then may be eaten by larger zooplankton, providing a previously unrecognized trophic link between small phytoplankton and large zooplankton. McManus and Ederington-Cantrell (1992) evaluated selective grazing of microzooplankton within the Chesapeake Bay using measurement of taxon-specific pigments. Such analyses of algal pigments, coupled with microscopic enumeration, provide information concerning microzooplankton distribution and trophic interactions.

## Historical Characterization

Identification of diagnostic pigments in suspended particulate matter allows for characterization of organic matter sources. Because analyses are relatively quick, individual Chls and carotenoids are separated prior to detection, and important processing indicators (e.g., pheophorbide and pheophytin) can be quantified, HPLC-based pigment analyses offer substantial advantages over spectrophotometric, fluorometric, and thin-layer chromatographic analyses previously used for paleolimnology (cf. Sanger and Gorham 1972; Zullig 1981, 1989; Swain 1985). Consequently, HPLC methodologies have become increasingly routine for estimating planktonic pigment delivery to and incorporation within both lacustrine (Leavitt et al. 1989; Hurley and Armstrong 1990, 1991; Hurley et al. 1992) and marine (Furlong and Carpenter 1988) sediments.

Accurate assessment of sedimentary pigments can allow the opportunity to compare long-term dynamics in phytoplankton composition. The presence or absence of diagnostic pigments is used for reconstruction of water-column phytoplankton history. However, a major complication of direct interpretation of sedimentary pigments arises from the fact that pigment production in the water column and sedimentation are not directly related. Water-column processes, such as photooxidation, cell lysis, and grazing, may preferentially degrade pigments prior to their incorporation within sediments (Repeta and Gagosian 1984; Carpenter et al. 1986; Hurley and Armstrong 1991). Zooplankton processing, in addition to transforming Chl to pheophorbide, may preferentially transport undegraded carotenoids to the sediment surface (Leavitt et al. 1989; Leavitt and Carpenter 1990). As a consequence, carotenoid relative abundance within sediments may be an unreliable measure of phytoplankton community composition. Rather, absolute concentrations, scaled to the historical maximum, should be used for fossil interpretations (see Leavitt 1993). Leavitt (1993) noted that three "phases" of Chl and carotenoid loss exist: (1) rapid oxidation in the water column, (2) slower postdepositional loss in the surface sediments, and (3) very slow loss of double bonds in deep sediments, losses that occur over temporal scales of days, years, and centuries, respectively. Such knowledge of the extent of processing pathways is essential prior to interpretation of pigment distribution within sediment cores.

Pigment analyses of sediment samples using HPLC can provide a significantly greater amount of information over traditional methods of pigment quantification. However, interpretation is hampered by the complexity of chromatograms. Pigment chromatograms from water-column samples typically contain 10–30 peaks where chromatograms from sediment samples often contain greater than 200 peaks (Repeta and Gagosian 1987). The ability to confirm individual packs from sediment samples usually is more complicated than those from water-column samples, and the ability to clearly interpret profiles is confounded by the susceptibility of individual pigments to diagenetic processes. For example, Hurley and Armstrong (1990) noted that peridinin was the most important carotenoid in the water column of Lake Mendota, Wisconsin (USA), during 1987, yet this pigment was not quantifiable in samples from sediment traps or surface sediments. Similarly, fucoxanthin, an important water-column pigment in northern Wisconsin (USA) lakes (Hurley and Armstrong 1991), was substantially degraded prior to incorporation in sediments, while diatoxanthin, a minor water-column pigment, was nearly completely retained during sedimentation. Repeta (1989) reported that postdeposition degradation of carotenoids in marine sediments is rapid and proportional to their ability to form unstable β-cyclic furanoxides. If environmental and ecological factors controlling degradation rates of pigments can be quantified, diagenetic models also may be used to reconstruct unaltered pigment profiles (Hurley et al. 1992). In addition, paleolimnological investigations which couple pigment analyses with microscopic analyses of plant pollen, bacillariophyte frustules, and zooplankton allow for better characterization of microalgal population dynamics concurrent with shifts in land use and/or anthropogenic activity. Such an approach could be expanded for assessing large-scale influences such as global climate change.

## Remote Sensing Applications

The recent purported global "epidemic" of toxic and noxious phytoplankton blooms in nutrient-enriched waters has

necessitated the use of satellite/airborne remote sensing for monitoring and potentially predicting bloom distribution. Toxic and noxious phytoplankton blooms often are monospecific and reach extremely high biomass values; yet blooms can be initiated and proliferated in subtle ways, at times escaping detection until more ominous symptoms occur (see Paerl 1988). HPLC–PDAS can serve a confirmatory role in "ground truthing" (or "sea truthing") the systematic significance of specific pigments and pigment groups for the remote sensing of algal reflectance "signatures" in a variety of aquatic systems.

The estimation of phytoplankton biomass by remote sensing technology relies upon the effects of pigments on algal spectral reflectance, which largely affects water color. Current practice relies upon Chl *a* as an estimate of algal biomass and has assumed cells to be homogeneous with respect to absorptive properties (Morel and Bricaud 1980). However, microalgae often use carotenoids (Haxo 1985; Owens et al. 1987) or phycobilins (Jones and Myers 1965; Stanier and Cohen-Bazire 1977) rather than Chls as the primary light-harvesting pigments. Additionally, many bloom-forming taxa can vary their pigment "signatures" in response to environmental conditions. For example, surface scums of the freshwater cyanophytes *Microcystis aeruginosa* Kuetz. and *Oscillatoria agardhii* Gomont often occur during periods of water-column stability in nutrient-enriched waters. The increased irradiance and UV radiation absorbed by these assemblages result in distinct lipophilic and phycobilin pigment modifications (Paerl et al. 1983, 1985; Millie et al. 1990b), thereby affecting cell-specific absorption coefficients (see Morel and Bricaud 1981) and, consequently, spectral reflectance "signatures." Such variability in the pigment "signature" of phytoplankton must be considered for accurate remote sensing.

The continued development of high-resolution sensors will evoke improved multispectral pigment scanning capabilities, thereby facilitating the acquisition of spectral reflectance data based upon pigments other than Chl *a* (see Millie et al. 1992a). To accomplish this, confirmation of accessory Chls and diagnostic carotenoids and the precise characterization of their absorption spectra by HPLC–PDAS will be required. However, the extraction of lipophilic pigments into organic solvents for HPLC analysis may shift the absorption characteristic of a particular pigment from as little as 6–16 nm for Chls to as great as 40–43 nm for fucoxanthin and its derivatives (Mann and Myers 1968; Haxo 1985; Bidigare et al. 1989b, 1990c; Millie et al. 1993). Consequently, in vivo absorption spectra are needed to accurately characterize the light-harvesting spectra of algae. Broad absorption bands within such spectra often occur, making absorption peaks difficult to interpret. Therefore, second-or fourth-derivative analyses are required to resolve the position of the absorption peaks and to assist in differentiating the shifts of absorption peaks between in vivo and extracted pigments (e.g., Faust and Norris 1985; Owens et al. 1987; Bidigare et al. 1989a; Millie et al. 1993). However, "pigment package" effects may be minimal for natural assemblages of open-oceanic phytoplankton at spectral regions where cellular absorption is high (i.e., Chl *a* absorption bands located near 440 and 675 nm; Bidigare et al. 1990c) or the red fluorescence signal of the cell (determined through flow cytometry) is proportional to the Chl content (Veldhuis and Kraay 1990).

The utility of passive remote sensing for distinguishing distinct pigments will be difficult, particularly in turbid waters where suspended sediments and dissolved organic matter increase the attenuation coefficient of water (Sathyendranath 1987) and accurate estimations of the spectra of back-scattered light are needed to distinguish phytoplankton pigments, non-pigmented particles, and dissolved organic matter (Klemas 1984; Sathyendranath et al. 1989). The broad-band sensors previously or currently used do not posses the radiometric sensitivity needed to distinguish individual pigments (see Millie et al. 1993). Spectrophotometers that collect passive imagery in nearly contiguous narrow spectral bands or active laser sensors which measure laser-induced in vivo fluorescence of Chl *a* and phycobilins may be more appropriate. In either event, with the deployment of high-resolution sensors such as SeaWIFS, OCTS, and Meris (in 1994, 1997, and 1998, respectively; Aiken et al. 1992), the coupling of remotely sensed imagery with accurate absorption spectra and HPLC-confirmed phytoplankton pigment "signatures" will be required to provide a previously unrecognized calibration of spectral reflectance "signatures" (e.g., Millie and Kirkpatrick 1992). Only then can the utility of remote sensing for estimating the spatial/temporal dynamics of phytoplankton community structure and production on local, regional, and global scales be accurately assessed. Additionally, the use of remotely sensed environmental information is key to characterizing hydrographic features and physical/chemical forcing events conducive to bloom initiation, maintenance, and transport (see Tyler and Stumpf 1989; Tester et al. 1991).

## Local, Regional, and Global Applications

The qualitative and quantitative detection and assessment of algal pigments will increase in frequency and importance as the biogeochemical effects of enhanced primary production, eutrophication, and associated nuisance algal blooms span from local to global scales. Cross-ecosystem interactions such as impacts of terrigenous discharge and atmospheric deposition of nutrients on enhancement of primary production in estuarine and coastal waters are examined very effectively by pigment analyses (Paerl et al. 1993a, 1993b).

Evaluation of the effects of nutrient and toxic inputs on synoptic and regional scales will require technology capable of dealing with samples from numerous locations, often at frequent sampling intervals. To this end, HPLC is very amenable to large-scale monitoring programs, such as the National Science Foundation's Joint Global Ocean Flux Studies (JGOFS) or the U.S. Environmental Protection Agency's Environmental Monitoring Assessment Program (EMAP). When coupled with auto-injectors and driven by state-of-the-art computer software, HPLC will be particularly useful for simultaneously determining pigment concentration and diversity among a large number of samples within a reasonable time frame. This approach greatly enhances the investigator's ability to accurately assess environmental impacts on primary production and community profiles on large scales (see Fig. 1), such as estuaries, lakes, and large segments of coastal and pelagic waters. A particularly well-suited application of this technology is the assessment of nutrient inputs, toxic discharges, and frontal passages (wetfall/dryfall) as they affect microalgal dynamics and production from the upper reaches of rivers and estuaries to the open waters of lakes and oceans (e.g., Cloern 1991; Rudek et al. 1991; Schaub and Gieskes 1991; Klarer and Millie 1992, 1994; Bianchi et al. 1993).

The key ingredient for detection and interpretation of remotely sensed pigments is accurate "ground truthing," capable of integration and intercalibration with remotely sensed imagery over

temporal and spatial scales analogous to biotic and abiotic processes affecting phytoplankton dynamics (e.g. Tester et al. 1991; Aiken et al. 1992; Harding et al. 1992; Millie et al. 1992a). For example, enhanced nutrient loading (in large part attributable to atmospheric deposition) has been linked to the stimulation of nuisance/toxic blooms, including the cyanophytes *Nodularia spumigina* Mertens and *Aphanizomenon flos-aquae* (L.) Ralfs in the Baltic Sea and the prymnesiophyte *Chyrsochromulina polylepsis* Manton et Parke in Norwegian coastal waters (Paerl 1993). Because phylogenetic distinctions among many taxa can be based upon distinct pigment and reflectance "signatures," HPLC and remote-sensing technologies can be useful in distinguishing individual blooms, both spatially and temporally, in response to specific physical/chemical forcing events, such as storm-water inputs, altered circulation patterns, and upwelling (Ulbricht and Horstmann 1980; Johannessen et al. 1989), as well as forecasting such blooms.

Passive and active remote-sensing technologies only are capable of imaging the surface and near-surface layer of the water column (approximately the top 22% of the total euphotic zone accounts for 90% of the signal for a passive sensor and active sensors sample a much smaller volume of the water column than passive sensors; Sathyendranath 1987). Consequently, statistical models linking surface remotely sensed pigments to total water-column pigments are needed to estimate total water-column production (see Platt and Herman 1983; Platt 1986; Smith et al. 1987). However, such models may severely underestimate true total production in instances where phytoplankton blooms occur deep within the water column (e.g., Borstad and Gower 1984; Bidigare et al. 1990b; Latasa et al. 1992; Hurley and Garrison 1993). As such, intensive "ground truthing" of phytoplankton pigments throughout the water column is necessary for accurate estimations of remotely sensed microalgal biomass and water-column production.

## Supplemental Technology

Recent technological advances have provided scientists with fiber optics and flow cytometric techniques for pigment research. Fibers that connect a solid-state laser or light-emitting diode to an underwater flow cell and coupled to a photodiode or charge-coupled detector provide a means to (fluorometrically) quantify Chl *a* biomass in situ. However, the accuracy and reproducibility of such analyses using fiber-optic detectors remain to be proven.

Flow cytometry utilizes laser-induced light scattering and autofluorescence of individual plankton cells within whole-water samples and can provide reproducible data concerning phytoplankton cell sizing and distribution, physiological state, cell cycle progression/differentiation, and zooplankton grazing. Veldhuis and Kraay (1990) combined flow cytometric and HPLC-based pigment analyses to identify and explain picoplankton distribution in the subtropical North Atlantic. Two distinct trends concerning prochlorophyte pigment concentrations were noted: zeaxanthin content remained fairly constant over the euphotic zone, and "red-shifted" Chl *a* (divinyl Chl *a*) and Chl *b* content increased with decreased irradiance. Additionally, when coupled with immunofluorescent probes (e.g., DNA, RNA, cell surface probes), flow cytometry can provide immunocytochemical information (for a review, see Yentsch 1990). However, although this "particle-derived" technology appears to offer several advantages over traditional "bulk" pigment analysis (including rapid quantification of distinct cell types and characterization of changes in cellular size and in vivo fluorescence properties) and can be utilized in shipboard operations, the equipment costs and expertise required to operate and maintain equipment appear to limit its routine applicability for most monitoring programs.

## Analytical Evaluation

HPLC-based pigment analyses provide unambiguous determinations of Chls, Chl derivatives, and carotenoids at extremely low detection levels. However, although HPLC has proven to be a powerful technique for separating and characterizing microalgal pigments, questions concerning universal applicability of this technique remain to be answered, most notably, cost, quantification methodology, reproducibility among laboratories, and availability of calibration standards. For example, the Scientific Committee For Ocean Research (SCOR) Working Group 78 recommended that specific microalgal cultures with known pigment compositions be used as the source of pigment standards (see Wright et al. 1991). However, unless researchers obtain the exact same strains from the same culture depository and all strains are grown under identical culture conditions, significant variability in the pigment "signature" of a strain might be expected. It would be most practical to establish reference cultures (grown under constant environmental conditions in a single culture depository) from which subsequent pigment extracts would serve as a source of standards. Efforts are currently underway to establish large quantities of crystalline pigments (extracted from pure cultures) which are to be housed in a national depository and made available for intercalibration among laboratories (G. Collins, Quality Assurance Research Division, U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA, personal communication).

The broad applications of HPLC methodology in systematic, ecological, and physiological research require the establishment of standard sampling, preparatory, and analytical protocols. However, a universal HPLC protocol able to satisfy all researchers' needs (i.e., simple, low cost, and able to resolve all closely eluting pigments such as Chls $c_1$ and $c_2$, lutein and zeaxanthin, lutein and diatoxanthin, neoxanthin and 19′-hexanoyloxyfucoxanthin, etc.) is not very feasible. Rather, researchers will utilize a variety of protocols (e.g., Mantoura and Llewellyn 1983; Bidigare et al. 1985; Wright and Jeffrey 1987; Wright et al. 1991; Kraay et al. 1992; Van Heukelem et al. 1992) to satisfy distinct informational needs and equipment and expense limitations/requirements. Additionally, the variety of HPLC hardware commercially available does not allow for (practical) standardization of hardware and supporting computer software. However, intercalibration of pigment-resolving abilities, identification, and quantification is necessary and obtainable (for recommendations, see Millie et al. 1993). PDAS provides the capability of characterizing absorption spectra of eluted HPLC "peaks," but computer software developed to date does not allow quantification of such spectra. This type of information will allow appropriate selection of channels within future satellite- and aircraft-based sensors used for the remote sensing of algal assemblages.

Accurate quantification of water-soluble phycobilin pigments is necessary for many studies. Phycobilin pigments are quantified spectrophotometrically using standardized equations (e.g., Bennett and Bogorad 1973). To date, a suitable HPLC methodology for the separation and quantification of phycobilins from

natural assemblages does not exist (cf. Swanson and Glazer 1990). Because these pigments are indicative of many noxious/toxic taxa, research should be directed towards (the possibility of) establishing such protocols. Flow cytometric methodology (see above) has been utilized for quantifying phycoerythrin and allows for accurate and reproducible quantification of cell phycerythrin content through autofluorescence (e.g., Wood et al. 1985; Li and Wood 1988; Olson et al. 1988).

Accurate experimental and system research requires rapid and selective characterization of algae. Pigment analyses have proven to be an effective means for rapidly characterizing algal phylogenetic groups and potentially taxa. However, the choice of a particular methodology for pigment determinations should depend upon the type of information required. If only total Chl *a* concentrations are desired, simple spectrophotometric and fluorometric measurements may suffice. If distinctions among specific pigments and/or their degradation products or distinctions among phylogenetic groups (using diagnostic pigments) are required for assessments of biogeochemical flux, water quality, production potential, or trophic state (Fig. 1), HPLC–PDAS can provide the appropriate pigment characterization.

## Acknowledgments

This review arose from ideas and concepts developed in the workshop "Evaluations of Spectrophotometric, Fluorometric, and High Performance Liquid Chromatographic Methods for Algal Pigment Determinations in Aquatic Ecosystems" convened at Duke University, Durham, North Carolina (USA), on 4 August 1991. We express appreciation to the U.S. Environmental Protection Agency (the Monitoring Management Branch, Region II and the Office of Research and Development, Environmental Monitoring Systems Laboratory) for funding Hans Paerl and David Millie to convene the workshop and the workshop participants for their interest, dedication, and contributions. We thank David Klarer, Alan Lewitus, Claire Schelske, Robert Seagull, Patricia Tester, James Wee, and an anonymous reviewer for criticizing preliminary drafts of the manuscript and are indebted to Gary Collins, Gary Fahnenstiel, Gary Kleppel, and Patricia Tester for their advice and encouragement.

## References

AAKERMANN, T., AND S. LIAAEN-JENSEN. 1992. Pyrrhoxanthin, reisolation and absolute configuration. Phytochemistry 31: 1779–1782.

AIKEN, J., G.F. MOORE, AND P.M. HOOLIGAN. 1992. Remote sensing of oceanic biology in relation to global climate change. J. Phycol. 28: 579–590.

ARPIN, N., W.A. SVEC, AND S. LIAAEN-JENSEN. 1976. A new fucoxanthin-related carotenoid from *Coccolithus huxleyi*. Phytochemistry 15: 529–532.

BAFFORD, R.A., R.A. SEAGULL, S.-Y. CHUNG, AND D.F. MILLIE. 1993. Intracellular localization of the taste/odor metabolite, 2-methylisoborneol, in *Oscillatoria limosa* (Cyanophyta). J. Phycol. 29: 91–95.

BARLOW, R.G., R.F.C. MANTOURA, M.A. GOUGH, AND T.W. FILEMAN. 1993. Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep-Sea Res. II. 40: 459–477.

BEN-AMOTZ, A., AND M. AVRON. 1980. β-carotene, and dry algal meal production by commercial cultivation on *Dunaliella*, p. 603–610. *In* G. Shelef and C.J. Soeder [ed.] Algae biomass. Elsevier/North Holland-Biomedial Press, Amsterdam, The Netherlands.

BENNETT, A., AND L. BOGORAD. 1973. Complimentary chromatic adaption in a filamentous blue-green alga. J. Cell Biol. 58: 419–435.

BIANCHI, T.S., AND S. FINDLEY. 1991. Decomposition of Hudson Estuary macrophytes: photosynthetic pigment transformations and decay constants. Estuaries 14: 65–73.

BIANCHI, T.S., S. FINDLEY, AND R. DAWSON. 1993. Organic matter sources in the water column and sediments of the Hudson River Estuary: the use of plant pigments as tracers. Estuarine Coastal Shelf Sci. 36: 359–376.

BIANCHI, T.S., S. FINDLEY, AND D. FONTVIELLE. 1991. Experimental degradation of plant materials in Hudson River sediments: I. Heterotrophic transformation of plant pigments. Biogeochemistry 12: 171–187.

BIDIGARE, R.R. 1989. Photosynthetic pigment composition of the brown tide alga: unique chlorophyll and carotenoid derivatives, p. 57–75. *In* E.M. Cosper, V.M. Bricelj, and E.J. Carpenter [ed.] Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Lecture notes on coastal and estuarine studies. Springer-Verlag, Berlin.

BIDIGARE, R.R., T.J. FRANK, C. ZASTROW, AND J.M. BROOKS. 1986. The distribution of algal chlorophylls and their degradation products in the southern Ocean. Deep-Sea Res. 33: 923–937.

BIDIGARE, R.R., M.C. KENNICUTT II, AND J.M. BROOKS. 1985. Rapid determination of chlorophylls and their degradation products by high-performance liquid chromatography. Limnol. Oceanogr. 30: 432–435.

BIDIGARE, R.R., M.C. KENNICUTT II, M.E. ONDRUSEK, M.D. KELLER, AND R.R.L. GUILLARD. 1990a. Novel chlorophyll-related compounds in marine phytoplankton: distributions and geochemical implications. Energy Fuels 4: 653–657.

BIDIGARE, R.R., J. MARRA, T.D. DICKEY, R. ITURRIAGA, K.S. BAKER, R.C. SMITH, AND H. PAK. 1990b. Evidence for phytoplankton succession and chromatic adaptation in the Sargasso Sea during spring 1985. Mar. Ecol. Prog. Ser. 60: 113–122.

BIDIGARE, R.R., J.H. MORROW, AND D.A. KIEFER. 1989a. Derivative analysis of spectral absorption by photosynthetic pigments in the western Sargasso Sea. J. Mar. Res. 47: 323–341.

BIDIGARE, R.R., M.E. ONDRUSEK, M.C. KENNICUTT II, R. ITURRIAGA, H.R. HARVEY, R.W. HOHAM, AND S. MACKO. 1993. Evidence for photoprotective function for secondary carotenoids of snow algae. J. Phycol. 29: 427–434.

BIDIGARE, R.R., M. ONDRUSEK, J.H. MORROW, AND D.A. KIEFER. 1990c. In vivo absorption properties of algal pigments. Proc. Soc. Photo/Opt. Instrum. Eng. 1302: 290–302.

BIDIGARE, R.R., O. SCHOFIELD, AND B.B. PRÉZLIN. 1989b. Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). Mar. Ecol. Prog. Ser. 56: 177–188.

BJØRNLAND, T. 1989. Carotenoid structure and lower plant phylogeny, p. 21–32. *In* N.I. Krinsky, M.M. Mathews-Roth, and R.F. Taylor [ed.] Carotenoids: chemistry and biology. Plenum Press, New York, N.Y.

BJØRNLAND, T., R.R.L. GUILLARD, AND S. LIAAEN-JENSEN. 1988. *Phaeocystis* sp. clone 677–3 — a tropical marine planktonic prymneisiophyte with fucoxanthin and 19′-acyloxyfucoxanthins as chemosystematic carotenoid markers. Biochem. Syst. Ecol. 16: 445–452.

BJØRNLAND, T., AND S. LIAAEN-JENSEN. 1989. Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics, p. 37–60. *In* J.C. Green, B.S.C. Leadbeater, and W.L. Diver [ed.] The chromophyte algae: problems and perspectives. Clarendon Press, Oxford, U.K.

BORSTAD, G.A., AND J.F.R. GOWER. 1984. Phytoplankton chlorophyll distribution in the eastern Canadian arctic. Arctic 37: 224–233.

BOWLES, N.D., H.W. PAERL, AND J. TUCKER. 1985. Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determinations. Can. J. Fish. Aquat. Sci. 42: 1127–1131.

BROWN, L.M., B.T. HARGRAVE, AND M.D. MACKINNON. 1981. Analysis of chlorophyll *a* in sediments by high-pressure liquid chromatography. Can. J. Fish. Aquat. Sci. 38: 205–214.

BUMA, A.G.J., N. BANO, M.J.W. VELDHUIS, AND G.W. KRAAY. 1991. Comparison of the pigmentation of two strains of the prymnesiophyte *Phaeocystis* sp. Neth. J. Sea Res. 27: 173–182.

CARPENTER, S.R., M.M. ELSER, AND J.J. ELSER. 1986. Chlorophyll production, degradation, and sedimentation: implications for paleolimnology. Limnol. Oceanogr. 31: 112–124.

CHAPMAN, D.J. 1966. Three new carotenoids isolated from algae. Phytochemistry 5: 1331–1333.

CLOERN, J.E. 1991. Annual variations in river flow and primary production in the South San Francisco Bay Estuary (USA), p. 91–96. *In* M. Elliot and J.-P. Ducrotoy [ed.] Estuaries and coasts: spatial and temporal intercomparisons. ECSA 19 Symposium, University of Caen, France, 4–8 September 1989. Olsen & Olsen, Fredensborg, Denmark.

CURACO, N., AND A.H. PUCCOON. 1986. The measurement of bacterial chlorophyll and algal chlorophyll *a* in natural waters. Limnol. Oceanogr. 31: 889–893.

EPPLEY, R.W., AND B.J. PETERSON. 1979. Particulate organic matter flux and planktonic new production in the deep ocean. Nature (Lond.) 228: 677–680.

EVERITT, D.A., S.W. WRIGHT, J.K. VOLKMAN, D.P. THOMAS, AND E.J. LINDSTROM. 1990. Phytoplankton community composition in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distributions. Deep-Sea Res. 37: 975–997.

FAUST, M.A., AND K.H. NORRIS. 1985. *In vivo* spectrophotometric analysis of photosynthetic pigments in natural populations of phytoplankton. Limnol. Oceanogr. 30: 1316–1322.

FAWLEY, M.W. 1991. Disjunct distribution of the xanthophyll loroxanthin in the green algae (Chlorophyta). J. Phycol. 27: 544–548.

FAWLEY, M.W. 1992. Photosynthetic pigments of *Pseudoscourfieldia marina* and select green flagellates and coccoid ultraphytoplankton: implications for the systematics of the Micromonadophyceae (Chlorophyta). J. Phycol. 28: 26–31.

FAWLEY, M.W., AND C.M. LEE. 1990. Pigment composition of the scaly green flagellate *Mesostigma viride* (Micromonadophyceae) is similar to that of the siphonous green alga *Bryopsis plumosa* (Ulvophyceae). J. Phycol. 26: 666–670.

FOSS, P., R.R.L. GUILLARD, AND S. LIAAEN-JENSEN. 1984. Prasinoxanthin — a chemosystematic marker for algae. Phytochemistry 23: 1629–1633.

FOSS, P., R.A. LEWIN, AND S. LIAAEN-JENSEN. 1987. Carotenoids of *Prochloron* sp. (Prochlorophyta). Phycologia 26: 142–144.

FURLONG, E.G., AND R. CARPENTER. 1988. Pigment remineralization in toxic marine sediments. Geochim. Cosmochim. Acta. 52: 87–89.

GARCIA-PICHEL, F., AND R.W. CATENHOLZ. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J. Phycol. 27: 395–409.

GEIDER, R.J., J. LAROCHE, R.M. GREEN, AND M. OLAIZOLA. 1993. Response of the photosynthetic apparatus of *Phaeodactylum tricornatum* (Bacillariophyceae) to nitrate, phosphate, or iron starvation. J. Phycol. 29: 755–766.

GIESKES, W.W. 1991. Algal pigment fingerprints: clue to taxon-specific abundance, productivity, and degradation to phytoplankton in seas and oceans, p. 61–99. *In* S. Demers [ed.] Particle analysis in oceanography. NATO ASI Series. Vol. G27. Springer-Verlag, Berlin.

GIESKES, W.W.C., M.M. ENGELKES, AND G.W. KRAAY. 1991. Degradation of diatom chlorophyll to colourless, non-fluorescing compounds during copepod grazing. Hydrobiol. Bull 25: 65–72.

GIESKES, W.W., AND G.W. KRAAY. 1982. Comparison of chromatographic (HPLC and TLC) conventional methods for the measurement of chlorophylls in oceanic waters. Arch. Hydrobiol. Beih. Ergebn. Limnol. 16: 123.

GIESKES, W.W., AND G.W. KRAAY. 1983a. Dominance of cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. Mar. Biol. 75: 757–766.

GIESKES, W.W.C., AND G.W. KRAAY. 1983b. Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. Limnol. Oceanogr. 28: 757–766.

GIESKES, W.W.C., AND G.W. KRAAY. 1984. Phytoplankton, its pigments, and primary production at a central North Sea station in May, July and September 1981. Neth. J. Sea Res. 18: 51–70.

GIESKES, W.W., AND G.W. KRAAY. 1986a. Floristic and physiological differences between the shallow and deep nanophytoplankton community in the eutrophic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. Mar. Biol. (Berl). 91: 567–576.

GIESKES, W.W.C., AND G.W. KRAAY. 1986b. Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the microflagellate *Corymbellus aureus* during the spring bloom in the open northern North Sea in 1983. Mar. Biol. 92: 45–52.

GIESKES, W.W.C., AND G.W. KRAAY. 1989. Estimating the carbon-specific growth rate of the major algal species groups in eastern Indonesian waters by $^{14}C$ labeling of taxon-specific carotenoids. Deep-Sea Res. 36: 1127–1139.

GIESKES, W.W.C., G.W. KRAAY, A. NONTJI, D. SETIAPERMANA, AND SUTOMO. 1988. Monsoonal alteration of a mixed and layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprints. Neth. J. Sea Res. 22: 123–137.

GOERICKE, R. 1992. The chlorophyll-labeling method: the radiochemical purity of chlorophyll *a* — a response to Jespersen et al. 1992, J. Plankton Res. 14: 1781–1785.

GOERICKE, R., AND D.J. REPETA. 1992. The pigments of *Prochlorococcus marinus*: the presence of divinyl chlorophyll *a* and *b* in a marine procaryote. Limnol. Oceanogr. 37: 425–433.

GOERICKE, R., AND N.A. WELSCHMEYER. 1992a. Pigment turnover in the marine diatom, *Thalassiosira weissflogii*. I. The $^{14}CO_2$-labeling kinetics of chlorophyll *a*. J. Phycol. 28: 498–507.

GOERICKE, R., AND N.A. WELSCHMEYER. 1992b. Pigment turnover in the marine diatom, *Thalassiosira weissflogii* II. The $^{14}CO_2$-labeling kinetics of carotenoids. J. Phycol. 28: 507–517.

GOERICKE, R., AND N.A. WELSCHMEYER. 1993a. The chlorophyll-labeling method: measuring specific rates of chlorophyll *a* synthesis in cultures and in the open ocean. Limnol. Oceanogr. 38: 80–95.

GOERICKE, R., AND N.A. WELSCHMEYER. 1993b. The carotenoid-labeling method: measuring specific rates of carotenoid synthesis in natural phytoplankton communities. Mar. Ecol. Prog. Ser. 98: 157–171.

GOWEN, R.J., P. TETT, AND B.J.B. WOOD. 1983. Changes in the major dihydroporphyrin plankton pigments during the spring bloom of the phytoplankton in two Scottish sealochs. J. Mar. Biol. Assoc. U.K. 63: 27–36.

GUILLARD, R.R.L., M.D. KELLER, C.J. O'KELLY, AND G.L. FLOYD. 1991. *Pycnococcus provasolii* gen. et sp. nov., a coccoid prasinoxanthin-containing phytoplankter from the western North Atlantic and Gulf of Mexico. J. Phycol. 27: 39–47.

GUILLARD, R.R.L., L.S. MURPHY, P. FOSS, AND S. LIAAEN-JENSEN. 1985. *Synechococcus* spp. as likely zeaxanthin-dominant ultraphytoplankton in the north Atlantic. Limnol. Oceanogr. 30: 412–414.

HARDING, L.W., JR., E.C. ITSWEIRE, AND W.E. ESAIAS. 1992. Determination of phytoplankton chlorophyll concentrations in the Chesapeake Bay with aircraft remote sensing. Remote Sens. Environ. 40: 79–100.

HAXO, F.T. 1985. Photosynthetic action spectrum of the coccolithophorid, *Emiliania huxleyi* (Haptophyceae): 19'hexanoyloxyfucoxanthin as antenna pigment. J. Phycol. 21: 282–287.

HEAD, E.J.H., AND L.R. HARRIS. 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar. Ecol. Prog. Ser. 86: 229–238.

HERTZBERG, S., AND S. LIAAEN-JENSEN. 1966a. The carotenoids of blue-green algae — I. The carotenoids of *Oscillatoria rubescens* and an *Athrospira* sp. Phytochemistry 5: 557–563.

HERTZBERG, S., AND S. LIAAEN-JENSEN. 1966b. The carotenoids of blue-green algae — II. The carotenoids of *Aphanizomenon flos-aquae*. Phytochemistry 5: 565–570.

HERTZBERG, S., AND S. LIAAEN-JENSEN. 1969a. The structure of myxoxanthophyll. Phytochemistry 8: 1259–1280.

HERTZBERG, S., AND S. LIAAEN-JENSEN. 1969b. The structure of oscillaxanthin. Phytochemistry 8: 1281–1292.

HERTZBERG, S., AND S. LIAAEN-JENSEN, AND H.W. SIEGELMAN. 1971. The carotenoids of blue-green algae. Phytochemistry 10: 3121–3127.

HOLM-HANSEN, O., C.J. LORENZEN, R.W. HOLMES, AND J.D.H. STRICKLAND. 1965. Flurometric determination of chlorophyll. J. Cons. perm. int. Explor. Mer 30: 3–15.

HOOKS, C.E., R.R. BIDIGARE, M.D. KELLER, AND R.R.L. GUILLARD. 1988. Coccoid eukaryotic marine ultraplankters with four different HPLC pigment signatures. J. Phycol. 24: 571–580.

HURLEY, J.P., AND D.E. ARMSTRONG. 1990. Fluxes and transformations of aquatic pigments in Lake Mendota, Wisconsin. Limnol. Oceanogr. 35: 384–398.

HURLEY, J.P., AND D.E. ARMSTRONG. 1991. Pigment preservation in lake sediments: a comparison of sedimentary environments in Trout Lake, Wisconsin. Can. J. Fish. Aquat. Sci. 48: 472–486.

HURLEY, J.P., D.E. ARMSTRONG, AND A.L. DUVALL. 1992. Historical interpretation of pigment stratigraphy in Lake Mendota sediments, p. 49–68. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y.

HURLEY, J.P., AND P.J. GARRISON. 1993. Composition and sedimentation of aquatic pigments associated with deep plankton in lakes. Can. J. Fish. Aquat. Sci. 50. (In press).

HURLEY, J.P., AND C.J. WATRAS. 1991. Identification of bacteriochlorophylls in lakes via reverse-phase HPLC. Limnol. Oceanogr. 36: 307–315.

JACOBSEN, T.R., AND H. RAI. 1990. Comparison of spectrophotometric, fluorometric and high performance liquid chromatography methods for determination of chlorophyll *a* in aquatic samples: effects of solvent and extraction procedures. Int. Rev. Gesamten Hydrobiol. 76: 207–217.

JEFFREY, S.W. 1961. Paper-chromatographic separation of chlorophylls and carotenoids from marine algae. Biochem. J. 80: 336–342.

JEFFREY, S.W. 1965. Paper chromatographic separation of pigments in marine phytoplankton. Aust. J. Mar. Freshwater. Res. 16: 307–313.

JEFFREY, S.W. 1968. Quantitative thin-layer chromatography of chlorophylls and carotenoids from marine algae. Biochim. Biophys. Acta 162: 271–285.

JEFFREY, S.W. 1974. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Mar. Biol. 26: 101–110.

JEFFREY, S.W. 1976. The occurrence of chlorophyll $c_1$ and $c_2$ in algae. J. Phycol. 12: 349–354.

JEFFREY, S.W. 1980. Algal pigment systems, p. 33–57. *In* P.G. Falkowski [ed.] Primary productivity in the sea. Plenum Press, New York, N.Y.

JEFFREY, S.W. 1981. An improved thin-layer chromatographic technique for marine phytoplankton pigments. Limnol. Oceanogr. 26: 191–197.

JEFFREY, S.W. 1989. Chlorophyll *c* pigments and their distribution in the chromophyte algae, p. 13–36. *In* J.C. Green, B.S.C. Leadbeater, and W.L. Diver [ed.] The chromophyte algae: problems and perspectives. Clarendon Press, Oxford, U.K

JEFFREY, S.W., AND G.M. HALLEGRAEFF. 1987. Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar. Ecol. Prog. Ser. 345: 293–304.

JEFFREY, S.W., M. SIELICKI, AND F.T. HAXO. 1975. Chloroplast pigment patterns in dinoflagellates. J. Phycol. 11: 374–385.

JEFFREY, S.W., AND M. VESK. 1976. Further evidence for a membrane-bound endosymbiont within the dinoflagellate *Peridinium foliaceum*. J. Phycol. 12: 450–455.

JESPERSEN, A.-M., J. NIELSEN, B. REIMANN, AND M. SONDERGAARD. 1992. Carbon-specific phytoplankton growth rates: a comparison of methods. J. Plankton Res. 14: 637–648.

JOHANNESSEN, J.A., O.M. JOHANNESSEN, AND P.M. HAUGAN. 1989. Remote sensing and model simulation studies of the Norwegian coastal current during the algal bloom in May 1988. Int. J. Remote Sens. 10: 1893–1906.

JOHNSEN, G., AND E. SAKSHAUG. 1993. Bio-optical characteristics and photoadaptive responses in the toxic and bloom-forming dinoflagellates *Gyrodinium aureolum, Gymnodinium galatheanum*, and two strains of *Prorocentrum minimum*. J. Phycol. 29: 627–642.

JONES, L.W., AND J. MYERS. Pigment variations in *Anacystis nidulans* induced by light of selected wavelengths. J. Phycol. 1: 7–15.

KLARER, D.M., AND D.F. MILLIE. 1992. Aquatic macrophytes and algae at Old Woman Creek Estuary and other Great Lakes coastal wetlands. *In* K.A. Kreiger, D.M. Klarer, R.T. Heath, and C.E. Herdendorf [ed.] Priorities for Great Lakes coastal wetlands research: a planning conference. J. Great Lakes Res. 18: 622–633.

KLARER, D.M., AND D.F. MILLIE. 1994. Regulation of phytoplankton dynamics in a Laurentian Great Lakes estuary. Hydrobiologia. (In press)

KLEIN, B., W.W.C. GIESKES, AND G.W. KRAAY. 1986. Digestion of chlorophylls and carotenoids by the marine protozoan *Oxyrrhis marina* studied by h.p.l.c. analysis of algal pigments. J. Plankton Res. 8: 827–836.

KLEIN, B., AND A. SOURNIA. 1987. A daily study of the diatom spring bloom at Roscoff (France) in 1985. II. Phytoplankton pigment composition studied by HPLC analysis. Mar. Ecol. Prog. Ser. 17: 265–275.

KLEMAS, V. 1984. Remote sensing of coastal and ocean properties. Proc. Soc. Photo-Opt. Instrum. Eng. 475: 42–54.

KLEPPEL, G.S., D. FRAZEL, R.E. PIEPER, AND D.V. HOLLIDAY. 1988. Natural diets of zooplankton off southern California. Mar. Ecol. Prog. Ser. 49: 231–241.

KLEPPEL, G.S., D.V. HOLLIDAY, AND R.E. PIEPER. 1991. Trophic interactions between copepods and microplankton: a question about the role of diatoms. Limnol. Oceanogr. 36: 172–178.

KLEPPEL, G.S., AND R.E. PIEPER. 1984. Phytoplankton pigments in the gut contents of planktonic copepods from coastal waters off southern California. Mar. Biol. 78: 193–198.

KOBAYASHI, M., T. KAKIZONO, AND S. NAGAI. 1993. Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cell of a green unicellular alga, *Haematococcus pluvialis*. Appl. Environ. Microbiol. 59: 867–873.

KOHATA, K., AND M. WATANABE. 1989. Diel changes in the composition of photosynthetic pigments and cellular carbon and nitrogen in *Pyramimonas parkeae* (Prasinophyceae). J. Phycol. 25: 377–385.

KRAAY, G.W., M. ZAPATA, AND M.J.W. VELDHUIS. 1992. Separation of chlorophylls $c_1$, $c_2$, and $c_3$ of marine phytoplankton by reversed-phase-C18-high-performance liquid chromatography. J. Phycol. 28: 708–712.

LATASA, M., M. ESTRADA, AND M. DELGADO. 1992. Plankton–pigment relationships in the northwestern Mediterranean during stratification. Mar. Ecol. Prog. Ser. 88: 61–73.

LEAVITT, P.R. 1993. A review of factors that regulate carotenoid and chlorophyll deposition and fossil pigment abundance. J. Paleolimnol. 9: 109–127.

LEAVITT, P.R., AND S.R. CARPENTER. 1990. Regulation of pigment sedimentation by photooxidation and herbivore grazing. Can. J. Fish. Aquat. Sci. 47: 1176–1176.

LEAVITT, P.R., S.R. CARPENTER, AND J.F. KITCHELL. 1989. Whole lake experiments: the annual record of fossil pigments and zooplankton. Limnol. Oceanogr. 34: 700–717.

LEGENDRE, L., AND M. GOSSELIN. 1989. New production and export of organic matter to the deep ocean: consequences of some recent discoveries. Limnol. Oceanogr. 34: 1374–1380.

LI, W.K.W., AND M. WOOD. 1988. Vertical distribution of North Atlantic ultraplankton: analysis by flow cytometry and epifluorescence microscopy. Deep-Sea Res. 35: 1615–1638.

LLOYD, S., AND C.S. TUCKER. 1988. Comparison of three solvent systems for extraction of chlorophyll a from fish pond phytoplankton communities. J. World Aquacult. Soc. 19: 36–40.

LORENZEN, C.J. 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea Res. 13: 223–227.

MANN, J.E., AND J. MEYERS. 1968. On pigments, growth, and photosynthesis of *Phaeodactylum tricornatum*. J. Phycol. 4: 349–355.

MANTOURA, R.F.C., AND C.A. LLEWELLYN. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. Anal. Chim. Acta 151: 297–314.

MCMANUS, G.B., AND M.C. EDERINGTON-CANTRELL. 1992. Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Mar. Ecol. Prog. Ser. 87: 77–85.

MILLIE, D.F., M.C. BAKER, C.S. TUCKER, B.T. VINYARD, AND C.P. DIONIGI. 1992a. High-resolution, airborne remote-sensing of bloom-forming phytoplankton. J. Phycol. 28: 281–290.

MILLIE, D.F., D.A. GREENE, AND P.B. JOHNSEN. 1990a. Effects of the carotenoid-inhibiting herbicide, fluridone, on *Oscillatoria agardhii* Gomont (Cyanobacteria). Aquat. Toxicol. 16: 41–52.

MILLIE, D.F., C.M. HERSH, AND C.P. DIONIGI. 1992b. Simazine-induced inhibition in photo-acclimated populations of *Anabaena circinalis* (Cyanophyta). J. Phycol. 28: 19–26.

MILLIE, D.F., D.A. INGRAM, AND C.P. DIONIGI. 1990b. Pigment and photosynthetic responses of *Oscillatoria agardhii* (Cyanophyta) to photon flux density and spectral quality. J. Phycol. 26: 660–666.

MILLIE, D.F., AND G.J. KIRKPATRICK. 1992. Are high-performance liquid chromatographic and remote-sensing technologies applicable for routine monitoring of *Gymnodinium breve* Davis? Paper presented at Aquatic Sciences Meeting, American Society of Limnology and Oceanography, 9–16 Feb., Santa Fe, N.M. (Abstr.)

MILLIE, D.F., H.W. PAERL, J.P. HURLEY, AND G.J. KIRKPATRICK. 1993. Algal pigment determinations in aquatic ecosystems: analytical evaluations, applications, and recommendations. *In* J. Menon [ed.] Current topics in botanical research. Council of Scientific Integration, Trvandrum, India (In press)

MOREL, A., AND A. BRICAUD. 1980. Theoretical results concerning the optics of phytoplankton, with special reference to remote sensing applications, p. 313–327. *In* J.F.R. Gower [ed.] Oceanography from space. Plenum Press, New York, N.Y.

MOREL, A., AND A. BRICAUD. 1981. Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. Deep-Sea Res. 28A: 1375–1393.

NAES, H., H.C. UTKILEN, AND A.F. POST. 1989. Geosmin production in the cyanobacterium *Oscillatoria brevis*. Arch. Microbiol. 151: 407–410.

NELSON, J.R. 1989. Phytoplankton pigments in macrozooplankton feces: variability in carotenoid alterations. Mar. Ecol. Prog. Ser. 52: 129–144.

NELSON, J.R. 1993. Rates and possible mechanism of light-dependent degradation of pigments in detritus derived from phytoplankton. J. Mar. Res. 1: 155–179.

O'CARRA, C., AND C. O'HEOCHA. 1976. Algal biliproteins and phycobilins, p. 328–376. *In* T.W. Goodwin [ed.] Chemistry and biochemistry of plant pigments. Vol. I. 2nd ed. Academic Press, London, U.K.

OLSON, R.J., S.W. CHISHOLM, E. ZETTLER, AND E.V. ARMBRUST. 1988. Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. Deep-Sea Res. 35: 425–440.

OWENS, T.G., J.C. GALLAGHER, AND R.S. ALBERTE. 1987. Photosynthetic light-harvesting function of violaxanthin in *Nannochloropsis* (Eustigmatophyceae). J. Phycol. 34: 79–85.

PAERL, H.W. 1984. Cyanobacterial carotenoids: their roles in maintaining optimal photosynthetic production among aquatic bloom forming genera. Oecologia (Berl.) 61: 143–149.

PAERL, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol. Oceanogr. 33: 823–847.

PAERL, H.W. 1993. Emerging role of atmospheric nitrogen deposition in coastal eutrophication: biogeochemical and trophic perspectives. Can. J. Fish. Aquat. Sci. 50: 2254–2269.

PAERL, H.W., B.M. BEBOUT, AND L.E. PRUFERT. 1989a. Naturally-occurring patterns of oxygenic photosynthesis and $N_2$ fixation in a marine microbial mat: physiologial and ecologial ramifications, p. 326–341. *In* Y. Cohen and E. Rosenberg [ed.] Microbial mats: physiological ecology of benthic microbial communities. ASM Publications, Washington, D.C.

PAERL, H.W., B.M. BEBOUT, L.E. PRUFERT, AND C.A. CURRIN. 1989b. Mediation of aquatic $N_2$ fixation potentials by $O_2$-depleted microzones, p. 113–117. *In* T. Hattori, Y. Ishida, Y. Maruyama, R.Y. Morita, and A. Ushida [ed.] Proceedings of the 5th International Symposium on Microbial Ecology, Kyoto, Japan, 28 August 1989. Japanese Science Society Press, Tokyo.

PAERL, H.W., P.T. BLAND, N.D. BOWLES, AND M.E. HAIBACH. 1985. Adaption to high-intensity, low-wavelength light among surface blooms of the cyanobacterium, *Microcystis aeruginosa*. Appl. Environ. Microbiol. 49: 1046–1052.

PAERL, H.W., M.L. FOGEL, AND P.W. BATES. 1993a. Atmospheric nitrogen

deposition in coastal waters: implications for marine primary production and carbon flux, p. 459–464. *In* C. Pedros-Alio [ed.] Proceedings of the 6th International Symposium on Microbial Ecology, Barcelona, Spain, September 1992. ASM Publications, Washington, D.C.

PAERL, H.W., M.L. FOGEL, P.W. BATES, AND P.M. O'DONNELL. 1993b. Is there a link between atmospheric nitrogen deposition and eutrophication in coastal waters? *In* K. Dyer [ed.] Changes in fluxes in estuaries and coastal waters. Proceedings of the 6th International ERF/ESCA Symposium, Plymouth, U.K., September 1992. Olsen and Olsen, Copenhagen. (In press)

PAERL, H.W., R.A. LEWIN, AND L. CHENG. 1984. Variations in chlorophyll and carotenoid pigmentation among *Prochloron* (Prochlorophyta) symbionts in diverse marine ascidians. Bot. Mar. 27: 257–264.

PAERL, H.W., J. TUCKER, AND P.T. BLAND. 1983. Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. Limnol. Oceanogr. 8: 847–857.

PALMISANO, A.C., R.E. SUMMONS, S.E. CRONIN, AND D.J. DES MARAIS. 1989. Lipophilic pigments from cyanobacterial (blue-green algal) and diatom mats in Hamelin Pool, Skark Bay, Western Australia. J. Phycol. 25: 655–661.

PARSONS, T.R., AND J.D. STRICKLAND. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations of ascertaining chlorophylls and carotenoids. J. Mar. Res. 21: 155–163.

PLATT, T. 1986. Primary production of the ocean water column as a function of surface light intensity: algorithms for remote sensing. Deep-Sea Res. 33: 149–163.

PLATT, T., AND A.W. HERMAN. 1983. Remote sensing of phytoplankton in the sea: surface layer chlorophyll as an estimate of water-column chlorophyll and primary production. Int. J. Remote Sens. 4: 343–351.

REDALJE, D.G. 1983. Phytoplankton carbon biomass and specific growth rates determined with the labeled chlorophyll technique. Mar. Ecol. Prog. Ser. 11: 217–225.

REDALJE, D.G. 1993. The labeled chlorophyll *a* technique for determining photoautotrophic carbon specific growth rates and carbon biomass, p. 563–572. *In* P.F. Kemp, E.F. Sherr, and J.J. Cole [ed.] Current methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Fla.

REDALJE, D.G., AND E.A. LAWS. 1981. A new method for estimating phytoplankton growth rates and carbon biomass. Mar. Biol. 62: 73–79.

REPETA, D.J. 1989. Early diagenesis of carotenoids in recent marine sediments. II. degradation of fucoxanthin to loliolides. Geochim. Cosmochim. Acta 53: 699–707.

REPETA, D.J., AND R.B. GAGOSIAN. 1984. Transformation reactions and recycling of carotenoids and chlorines in the Peru Upwelling region (15°S, 75°W). Geochim. Cosmochim. Acta 48: 1265–1277.

REPETA, D.J., AND R.B. GAGOSIAN. 1987. Carotenoid diagenesis in recent marine sediments — I. The Peru continental shelf (15°S, 75°W). Geochim. Cosmochim. Acta 51: 1001–1009.

REPETA, D.J., D.J. SIMPSON, B.B. JORGENSEN, AND H.W. JANNASCH. 1989. Evidence for anoxygenic photosynthesis from the distribution of bacteriochlorophylls in the Black Sea. Nature (Lond.) 342: 69–72.

RICHARDS, F.A., AND T.G. THOMPSON. 1952. The estimation and characterization of plankton populations by pigment analyses. II. A spectrophotometric method for estimation of plankton pigments. J. Mar. Res. 11: 156–171.

RICKETTS, T.R. 1966. Magnesium 2,4-divinylphaeoporphyrin $a_5$ monomethyl ester, a protochlorophyll-like pigment present in some unicellular flagellates. Phytochemistry 5: 223–229.

RICKETTS, T.R. 1970. The pigments of the prasinophyceae and related organisms. Phytochemistry 9: 1835–1842.

RIDOUT, P.S., AND R.J. MORRIS. 1985. Short-term variations in pigment composition of a spring phytoplankton bloom from an enclosed experimental system. Mar. Biol. 87: 7–11.

ROWAN, K.S. 1989. Photosynthetic pigments of algae. Cambridge University Press, Cambridge. 334 p.

RUDEK, J., H.W. PAERL, M.A. MALLIN, AND P.W. BATES. 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River estuary, North Carolina, Mar. Ecol. Prog. Ser. 75: 133–142.

SANGER, J.E., AND E. GORHAM. 1972. Stratigraphy of fossil pigments as a guide to the postglacial history of Kirchner Marsh, Minnesota. Limnol. Oceanogr. 17: 840–854.

SATHYENDRANATH, S. 1987. Remote sensing of phytoplankton: a review, with special reference to picoplankton. Can. Bull. Fish. Aquat. Sci. 214: 561–587.

SATHYENDRANATH, S., L. PRIEUR, AND A. MOREL. 1989. A three-component model of ocean colour and its application to remote sensing of phytoplankton pigments in coastal waters. Int. J. Remote Sens. 10: 1373–1394.

SCHAUB, B.E.M., AND W.W.C. GIESKES. 1991. Eutrophication of the North Sea: the relation between Rhine River discharge and the chlorophyll-*a* concentration in Dutch coastal waters, p. 85–90. *In* M. Elliot and J.-P. Ducrotoy [ed.] Estuaries and coasts: spatial and temporal intercomparisons. ECSA 19 Symposium, University of Caen, France, 4–8 September 1989. Olsen & Olsen, Fredensborg, Denmark.

SCHOCH, S., H. SCHEER, J.A. SCHIFF, W. RIDIGER, AND H.W. SIEGELMAN. 1981. Pyropheophytin *a* accompanies pheophytin *a* in darkened light grown cells of *Euglena*. Z. Naturforsch. 36: 827–833.

SHAISH, A., A. BEN-AMOTZ, AND M. AVRON. 1991. Production and selection of high β-carotene mutants of *Dunaliella bardawil* (Chlorophyta). J. Phycol. 27: 652–656.

SMITH, R.C., K.S. BAKER, AND P. DUSTAN. 1981. Fluorometric technique for the measurement of oceanic chlorophyll in the support of remote sensing. Rep. 81–17, Scripps Institute of Oceanography, University of California-San Diego, La Jolla, CA 92093, USA. 14 p.

SMITH, R.C., R.R. BIDIGARE, B. B. PRÉZELIN, K.S. BAKER, AND J.M. BROOKS. 1987. Optical characterization of primary productivity across a coastal front. Mar. Biol. 96: 575–591.

SOMA, Y., T. IMAIZUMI, K-I. YAGI, AND S-I. KASUGA. 1993. Estimation of algal succession in lake water using HPLC analysis of pigments. Can. J. Fish. Aquat. Sci. 50: 1142–1146.

STANIER, R.Y., AND G. COHEN-BAZIRE. 1977. Phototrophic procaryotes: the cyanobacteria. Annu. Rev. Microbiol. 31: 225–274.

STANSKY, H., AND A. HAGER. 1970. Des carotinoidmuster und die verbreitung des lichtinduzierten xanthophyll-cyclus in verschiedenen algenklassen. II. Xanthophyceae. Arch. Mikrobiol. 71: 132–163.

STAUBER, J.L., AND S.W. JEFFREY. 1988. Photosynthetic pigments in fifty one species of marine diatoms. J. Phycol. 24: 158–172.

STRAIN, H.H., W.A. SVEC, K. AITZETMÜLLER, M.C. GRANDOLFO, J.J. KATZ, H. KJØSEN, S. NORGARD, S. LIAAEN-JENSEN, F.T. HAXO, P. WEGFAHRT, AND H. RAPPAPORT. 1971. The structure of peridinin, the characteristic dinoflagellate carotenoid. J. Am. Chem. Soc. 93: 1823–1825.

STRICKLAND, J.D.H. 1968. Continuous measurement of *in vivo* chlorophyll: a precaution. Deep-Sea Res. 15: 225–227.

STRICKLAND, J.D.H., AND T.R. PARSONS. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 310 p.

STROM, S.L., AND N.A. WELSCHMEYER. 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnol. Oceanogr. 36: 50–63.

SWAIN, E.B. 1985. Measurement and interpretation of sedimentary pigments. Freshwater Biol. 15: 53–75.

SWANSON, R.V., AND A.N. GLAZER. 1990. Separation of phycobiliprotein subunits by reverse-phase high-pressure liquid chromatography. Anal. Biochem. 188: 295–299.

TANGEN, K., AND T. BJØRNLAND. 1981. Observation on pigments and morphology of *Gyrodinium aureolum* Hulbert, a marine dinoflagellate containing 19'hexanoyloxyfucoxanthin as the main carotenoid. J. Plankton Res. 3: 389–401.

TESTER, P.A., R.P. STUMPF, F.N. VUKOVICH, P.K. FOWLER, AND J.T. TURNER. 1991. An expatriate red tide bloom: transport, distribution, and persistence. Limnol. Oceanogr. 36: 1053–1061.

TOMAS, R.N., AND E.R. COX. 1973. Observations on the symbiosis of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. J. Phycol. 9: 304–323.

TREES, C.C., M.C. KENNICUTT II, AND J.M. BROOKS. 1985. Errors associated with the standard fluorometric determination of chlorophylls and pheopigments. Mar. Chem. 17: 1–12.

TYLER, M.A., AND R.P. STUMPF. 1989. Feasibility of using satellites for detection of kinetics of small phytoplankton blooms in estuaries: tidal and migrational effects. Remote Sens. Environ. 27: 233–250.

ULBRICHT, K.A., AND U. HORSTMANN. 1980. Remotely sensed phytoplankton development in the Baltic Sea, p. 69–76. *In* A.P. Cracknell [ed.] Coastal and marine applications of remote sensing. Remote Sensing Soc., Reading, England.

UTKILEN, H., AND N. GJØLME. 1992. Toxin production by *Microcystis aeruginosa* as a function of light in continuous culture. Appl. Environ. Microbiol. 58: 1321–1325.

VAN HEUKELEM, L., A.J. LEWITUS, T.M. KANA, AND N.E. CRAFT. 1992. High-performance liquid chromatography of phytoplankton pigments using a polymeric reversed-phase $C_{18}$ column. J. Phycol. 28: 867–872.

VELDHUIS, M.J.W., AND G.W. KRAAY. 1990. Vertical distribution and pigment composition of a picoplanktonic prochlorophyte in the subtropical North Atlantic: a combined study of HPLC-analysis of pigments and flow cytometry. Mar. Ecol. Prog. Ser. 68: 121–127.

WATANABE, M.M., S. SHOICHIRO, I. INOUYE, T. SAWAGUCHI, AND M. CHIHARA. 1990. *Lepidodinium viride* gen. et sp. nov. (Gymnodiniales, Dinophyta), a green dinoflagellate with a chlorophyll *a*- and *b*-containing endosymbiont. J. Phycol. 26: 741–751.

WELSCHMEYER, N.A., AND C.J. LORENZEN. 1985. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyre. Limnol. Oceanogr. 30: 1–21.

WILHELM, C., AND L. MANNS. 1991. Changes in pigmentation of phytoplankton species during growth in stationary phase — consequences for reliability of pigment-based methods of biomass determinations. J. Appl. Phycol. 3: 305–310.

WILHELM, C., I. RUDOLPH, AND W. RENNER. 1991. A quantitative method based on HPLC-aided pigment analysis to monitor structure and dynamics of the phytoplankton assemblage — a study from Lake Meerfelder (Eifel, Germany). Arch. Hydrobiol. 123: 21–35.

WITHERS, N.W., E.R. COX, R. TOMAS, AND F.T. HAXO. 1977. Pigments of the dinoflagellate *Peridinium balticum* and its photosynthetic endosymbiont. J. Phycol. 13: 354–358.

WOOD, A.M., P.K. HORAN, K. MUIRHEAD, D.A. PHINNEY, C.M. YENTSCH, AND J.B. WATERBURY. 1985. Discrimination between types of pigments in marine *Synechococcus* spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry. Limnol. Oceanogr. 30: 1303–1315.

WRIGHT, S.A., AND S.W. JEFFREY. 1987. Fucoxanthin pigment markers of marine phytoplankton analyzed by HPLC and HPTLC. Mar. Ecol. Prog. Ser. 38: 259–266.

WRIGHT, S.W., S.W. JEFFREY, R.F.C. MANTOURA, C.A. LLEWELLYN, T. BJØRNLAND, D. REPETA, AND N. WELSCHMEYER. 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser. 77: 183–196.

WRIGHT, S.A., AND J.D. SHEARER. 1984. Rapid extraction and high-performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. J. Chromatogr. 294: 281–295.

YENTSCH, C.M. 1990. Environmental health: flow cytometric methods to assess our water world. Methods Cell Biol. 33: 575–612.

YENTSCH, C.S., AND D.M. MENZEL. 1963. A method for the determination of phytoplankton chlorophyll and pheophytin by fluorescence. Deep-Sea. Res. 10: 221–231.

YENTSCH, C.S., AND C.M. YENTSCH. 1979. Fluorescence spectral signatures: the characterization of phytoplankton populations by the use of excitation and emission spectra. J. Mar. Res. 37: 471–483.

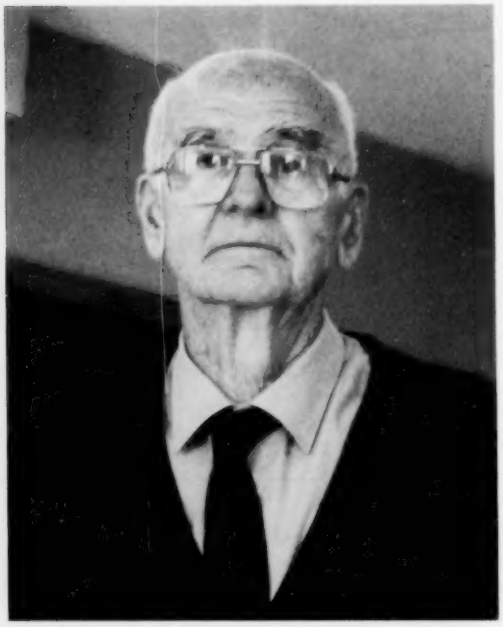
ZULLIG, H. 1981. On the use of carotenoid stratigraphy in lake sediments for detecting past developments of phytoplankton. Limnol. Oceanogr. 26: 970–976.

ZULLIG, H. 1989. Role of carotenoids in lake sediments for reconstructing trophic history during the late Quaternary. J. Paleolimnol. 2: 23–40.

# OBITUARY/NÉCROLOGIE

## *Daniel Branch Quayle*

### *1913–1993*



Daniel (Dan) Branch Quayle, a world authority in the field of molluscan biology and bivalve culture, passed away October 19, 1993, at his home in Nanaimo, British Columbia. He was 80 years old.

Born in the U.K. in 1913, Dan emigrated to Canada with his family when he was three years old. The family settled in Ladysmith, B.C., where his father worked in the local coal mines. Dan undoubtedly developed his love of marine life at an early age as he played around the shores of Ladysmith Harbour.

After attending elementary and secondary school in Ladysmith, he went to the Provincial Normal School in Victoria in 1929, graduating with his teaching certificate in 1930. For the next three years, he taught in the Michel-Natal area of south-eastern B.C. He maintained a love of young people and teaching for the rest of his life.

His love of the sea brought him back to the coast in 1934, where he enrolled at the University of British Columbia in Vancouver. He received his B.A. in Zoology in 1937. While at U.B.C., he worked as a summer student under Dr. C.R. Elsey and began his lifelong interest in oysters. He assisted Dr. Elsey with studies of Pacific oyster (*Crassostrea gigas*) breeding in Ladysmith Harbour. Together, they transplanted juvenile oysters from Ladysmith Harbour to a number of isolated inlets along the west coast of Vancouver Island in 1937. The transplants were successful and offspring can still be found in these inlets. He went on to receive his M.A. in Zoology at U.B.C. in 1938 under Dr. MacLean-Fraser.

In 1938, he began working for the Fisheries Research Board of Canada at the Pacific Biological Station in Nanaimo. His association with the Station would span a period of 55 years. He assumed responsibility for the molluscan research program and initiated an investigation of the molluscan resources of British Columbia. Although this work focused on species with commercial potential, he also began studies of the ecology of marine invertebrates. These studies would continue for the rest of his life.

His scientific work was interrupted by the onset of World War II. In 1941, he joined the Royal Canadian Air Force and became a navigator in an elite pathfinder squadron. He spent one and a half years as a prisoner of war after being shot down over Germany.

.He returned briefly to the Pacific Biological Station at the end of the war in 1945 before leaving shortly thereafter to begin Ph.D. studies at the University of Glasgow under the direction of Sir C.M. Yonge. After receiving his Ph.D. in 1948, Dan returned to Canada to begin work as Director of Biological Services for the B.C. Provincial Department of Fisheries. He built a small laboratory in Ladysmith and initiated extensive studies of oysters and oyster culture. During this time, he worked closely with industry, pioneering methods to improve oyster production. He maintained this close association with the oyster culture industry throughout his career.

Dan was Director of Fisheries for the Province of B.C. from 1956 to 1957. In 1957, he went to the United States to serve as a technical advisor to the Coast Oyster Company in South Bend, Washington, and a Chesapeake Bay oyster consortium in Virginia.

He returned to the Pacific Biological Station in 1958 and continued his study of the ecology of marine invertebrates until his retirement in 1973. He undertook extensive studies of marine invertebrate populations along the B.C. coast and became recognized as a world authority in the field. Dan maintained a keen interest in a wide range of marine invertebrate topics, publishing numerous scientific and semi-scientific papers. His book "Pacific Oyster Culture in British Columbia" became a standard text for oyster culture, particularly on the west coast of North America. In fact, much of the success of the B.C. oyster culture industry is due to the efforts of Dan Quayle.

After retiring from the Pacific Biological Station in 1973, Dan became a consultant for a number of Canadian overseas aid agencies, including the Canadian International Development Agency (CIDA), International Development Research Centre

(IDRC), and Canadian Executive Services Overseas (CESO). In this capacity, he travelled to developing countries all over the world to assist in the development of shellfish culture industries. The results of this work led to a number of publications which served as texts for bivalve culture in these countries. He also taught courses on oyster culture at Dalhousie University in Halifax, Nova Scotia.

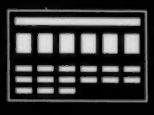
When not involved in overseas consulting, Dan was an active volunteer researcher at the Pacific Biological Station until shortly before his death. He continued his studies on marine invertebrates and published widely. A major revision of his publication "Pacific Oyster Culture in British Columbia" appeared in 1988 and, shortly before his death, he completed a major work entitled "Marine Wood Borers in British Columbia." In 1989, he received an honorary LL.D. degree from the University of Victoria in recognition of his work in the field of marine invertebrates and molluscan culture in B.C. and throughout the world.

Dan was an inspiration to the staff of the Pacific Biological Station and was highly respected for his never-ending quest for knowledge. He was a kind and patient person who always had time to talk to younger staff members, giving them the benefit of his long years of experience. He was a wonderful field and shipboard companion. Those of us who were privileged to travel with him will remember close companionship and long philosophical discussions on a wide variety of topics which have done much to stimulate, assist, and direct us in our work over the years.

Dan is survived by his wife Ann, his daughter Moura, and a host of friends whose lives have been enriched by their association with him.

N.F. Bourne
*Pacific Biological Station*
*Nanaimo, B.C.*

THIS PUBLICATION AVAILABLE FROM UMI



UMI

This publication is available from UMI in one or more of the following formats:

- **In Microform**--from our collection of over 18,000 periodicals and 7,000 newspapers
- **In Paper**--by the article or full issues through UMI Article Clearinghouse
- **Electronically, on CD-ROM, online, and/or magnetic tape**--a broad range of ProQuest databases available, including abstract-and-index, ASCII full-text, and innovative full-image format

Call toll-free 800-521-0600, ext. 2888, for more information, or fill out the coupon below:

Name _____

Title _____

Company/Institution _____

Address _____

City/State/Zip _____

Phone (  ) _____

I'm interested in the following title(s): _____

_____

_____

_____

**UMI**
**A Bell & Howell Company**
**Box 78**
**300 North Zeeb Road**
**Ann Arbor, MI 48106**
**800-521-0600 toll-free**
**313-761-1203 fax**

## CODATA

### Committee on Data for Science and Technology

A Scientific Committee of the International Council of Scientific Unions (ICSU), CODATA is concerned with all types of quantitative data resulting from experimental measurements or observations in the physical, biological, geological, and astronomical sciences. Particular emphasis is given to data management problems common to different scientific disciplines and to data used outside the field in which they were generated.

The general objectives of CODATA are the improvement of the quality and accessibility of data, as well as the methods by which data are acquired, managed, and analyzed; the facilitation of international cooperation among those collecting, organizing, and using data; and the promotion of an increased awareness in the scientific and technical community of the importance of these activities.

The Canadian National Committee for CODATA (CNC/CODATA), which provides liaison between Canadian scientists and engineers and CODATA, is anxious to involve as many interested individuals as possible in its endeavours. If you would like to know more about CODATA, become associated with CNC/CODATA, or receive the quarterly CODATA Newsletter, please contact

**Secretariat, CNC/CODATA**
**CISTI, Building M-55, Rm 275**
**National Research Council**
**Montreal Road**
**Ottawa, ON K1A 0S2**
**Telephone: 613-993-3294**
**Fax: 613-952-8246**

Do you have a bright idea that's being put to use in Canada? Know of anyone else who has? If so, the Ernest C. Manning Awards Foundation wants to hear from you now.

You can nominate someone for the 1994 Manning Awards, including a Principal Award of $100,000, a $25,000 Award of Distinction and two $5,000 Innovation Awards.

The idea may relate to virtually any aspect of Canadian life. It can be a new concept, process or product that's in use or development and of benefit to Canadians.

Nominations close **February 11, 1994.**

For your nomination form, call or write: THE MANNING AWARDS, 3900, 421-7 Ave. S.W., Calgary, Alberta T2P 4K9, Telephone: (403) 266-7571 FAX (403) 266-8154.

SALUTING CANADIAN INNOVATION

1994

Annual Subscription Rates / Abonnements annuels*†‡

| Publication | Volume | Code | Canada ($CAN) Institutional/ Collectif (CI) | Canada ($CAN) Personal/ Personnel (CP) | Foreign / Étranger ($US) Institutional/ Collectif (FI) | Foreign / Étranger ($US) Personal/ Personnel (FP) | Foreign / Étranger ($US) Air mail / Par avion Add / ajouter |
|---|---|---|---|---|---|---|---|
| Monthly / Mensuelle | | | | | | | |
| Biochemistry and Cell Biology / Biochimie et biologie cellulaire | 72 | 44 | 237 | 88 | 237 | 90 | 75 |
| Canadian Journal of Botany / Revue canadienne de botanique | 72 | 45 | 367 | 145 | 367 | 155 | 150 |
| Canadian Journal of Chemistry / Revue canadienne de chimie | 72 | 46 | 454 | 143 | 454 | 152 | 150 |
| Canadian Journal of Earth Sciences / Revue canadienne des sciences de la Terre | 31 | 47 | 365 | 117 | 365 | 123 | 150 |
| Canadian Journal of Fisheries and Aquatic Sciences / Journal canadien des sciences halieutiques et aquatiques | 51 | 261 | 320 | 122 | 360 | 135 | 150 |
| Canadian Journal of Forest Research / Revue canadienne de recherche forestière | 24 | 54 | 324 | 107 | 324 | 111 | 75 |
| Canadian Journal of Microbiology / Revue canadienne de microbiologie | 40 | 48 | 258 | 100 | 258 | 102 | 75 |
| Canadian Journal of Physics / Revue canadienne de physique | 72 | 49 | 285 | 94 | 285 | 96 | 75 |
| Canadian Journal of Physiology and Pharmacology / Revue canadienne de physiologie et pharmacologie | 72 | 50 | 333 | 110 | 333 | 115 | 75 |
| Canadian Journal of Zoology / Revue canadienne de zoologie | 72 | 51 | 401 | 152 | 401 | 163 | 150 |
| Bimonthly / Bimestrielle | | | | | | | |
| Canadian Journal of Civil Engineering / Revue canadienne de génie civil | 21 | 52 | 259 | 84 | 259 | 86 | 75 |
| Genome / Génome | 37 | 70 | 239 | 99 | 239 | 101 | 75 |
| Canadian Geotechnical Journal / Revue canadienne de géotechnique | 31 | 53 | 207 | 79 | 207 | 84 | 50 |
| Quarterly / Trimestrielle | | | | | | | |
| Environmental Reviews/ Dossiers environnement | 2 | 244 | 163 | 57 | 163 | 63 | 25 |

*Price of regular single issues of all journals: institutional, $43.00; personal, $19.00. / Prix des numéros réguliers de toutes les revues : collectif, 43,00 $; personnel, 19,00 $.

†Rates include second-class postage. Canadian customers please include additional 7% GST (GST No. R121491807). In Quebec please add a further 8% QST to the total price including GST (QST No. 1006178088). / Affranchissement de deuxième classe compris. Clients canadiens veuillez ajouter 7% pour la TPS (N° TPS R121491807). Au Québec veuillez ajouter un 8% de plus pour la TVQ au montant total incluant TPS (N° TVQ 1006178088).

‡Special rates apply for members of Canadian scientific and engineering societies if they subscribe through their respective societies. / Les tarifs spéciaux sont offerts aux membres des sociétés scientifiques et des sociétés de génie au Canada, s'ils s'abonnent par l'intermédiaire de leur société respective.

**Subscriptions, renewals, requests for single or back numbers, and all remittances** should be sent to Subscription Office, Research Journals, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada. Remittances should be made payable to the Receiver General for Canada, credit National Research Council of Canada. Enquiries: Helen Goulet 613-993-9084. Fax No. 613-952-7656.

Subscriptions are entered for the calendar year. There are no refunds on cancelled subscriptions once delivery has begun.

**Change of address** - Send change of address information (complete old address, customer code, and new address) to Subscription Office, Research Journals, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

**Claims for missing numbers** should be made within four months (North America) or six months (outside North America) following the date of publication. The publisher expects to supply missing numbers only when losses have been sustained in transit and when the reserve stock will permit.

**Abonnements - Pour toutes les questions d'abonnements, paiements, demandes de vieux numéros de la revue, etc.,** on doit s'adresser au Bureau d'abonnements, Revues scientifiques, Conseil national de recherches du Canada, Ottawa, ON, K1A 0R6, Canada. Les chèques et mandats seront formulés à l'ordre du Receveur général du Canada, au crédit du Conseil national de recherches du Canada. Renseignements : Helen Goulet 613-993-9084. N° de télécopieur 613-952-7656.

La durée des abonnements correspond à l'année civile. Les abonnements annulés ne pourront être remboursés une fois que le premier numéro sera envoyé.

**Changement d'adresse** - Faire parvenir tous renseignements (ancienne adresse, code du client et nouvelle adresse) au Bureau d'abonnements, Revues scientifiques, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

**Les réclamations de numéros manquants** doivent être adressées dans les quatre mois (en Amérique du Nord) ou les six mois (à l'étranger) suivant la date de publication. L'éditeur s'engage à remplacer les numéros manquants seulement lorsque les pertes ont été subies en transit et lorsque ses réserves le permettent.

## Reviews/Synthèses

**Millie, D.F., H.W. Paerl, and J.P. Hurley.** Microalgal pigment assessments using high-performance liquid chromatography: a synopsis of organismal and ecological applications **2513–2527**

## Obituary/Nécrologie

**Bourne, N.F.** Daniel Branch Quayle, 1913–1993 **2528–2529**

National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Postage paid at Ottawa
Publications mail
Registration pending

Port payé à Ottawa
Poste-publication
Enregistrement en cours

# Canadian Journal of Fisheries and Aquatic Sciences

# Journal canadien des sciences halieutiques et aquatiques

**Volume 50, No. 11, November 1993**

**Volume 50, n° 11, novembre 1993**

**Domm, S., R.W. McCauley, E. Kott, and J.D. Ackerman.** Physiological and taxonomic separation of two dreissenid mussels in the Laurentian Great Lakes **2294–2297**

**Dermott, R., and M. Munawar.** Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications **2298–2304**

**Mills, E.L., R.M. Dermott, E.F. Roseman, D. Dustin, E. Mellina, D.B. Conn, and A.P. Spidle.** Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes **2305–2314**

**Gislason, H.** Effect of changes in recruitment levels on multispecies long-term predictions **2315–2322**

**Heide-Jørgensen, M.P., H. Lassen, J. Teilmann, and R.A. Davis.** An index of the relative abundance of wintering belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, off West Greenland **2323–2335**

**Bevelhimer, M.S., and S.M. Adams.** A bioenergetics analysis of diel vertical migration by kokanee salmon, *Oncorhynchus nerka* **2336–2349**

**Kline, T.C. Jr., J.J. Goering, O.A. Mathisen, P.H. Poe, P.L. Parker, and R.S. Scalan.** Recycling of elements transported upstream by runs of Pacific salmon: II. $\delta^{15}N$ and $\delta^{13}C$ evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska **2350–2365**

**Jackson, G.D.** Growth zones within the statolith microstructure of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae): evidence for a habitat shift? **2366–2374**

**Maekawa, K., T. Hino, S. Nakano, and W.W. Smoker.** Mate preference in anadromous and landlocked Dolly Varden (*Salvelinus malma*) females in two Alaskan streams **2375–2379**

**Beecher, H.A., T.H. Johnson, and J.P. Carleton.** Predicting microdistributions of steelhead (*Oncorhynchus mykiss*) parr from depth and velocity preference criteria: test of an assumption of the Instream Flow Incremental Methodology **2380–2387**

**Somers, K.M., and D.A. Jackson.** Adjusting mercury concentration for fish-size covariation: a multivariate alternative to bivariate regression **2388–2396**

**Grewe, P.M., C.H. Krueger, C.F. Aquadro, E. Bermingham, H.L. Kincaid, and B. May.** Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario **2397–2403**

**Arts, M.T., R.D. Robarts, and M.S. Evans.** Energy reserve lipids of zooplanktonic crustaceans from an oligotrophic saline lake in relation to food resources and temperature **2404–2420**

**Paloheimo, J.E., and Y. Chen.** Estimation of effective effort from catch-at-age data **2421–2428**

**Stenson, G.B., R.A. Myers, M.O. Hammill, I.-H. Ni, W.G. Warren, and M.C.S. Kingsley.** Pup production of harp seals, *Phoca groenlandica*, in the northwest Atlantic **2429–2439**

**McKinley, R.S., T.D. Singer, J.S. Ballantyne, and G. Power.** Seasonal variation in plasma nonesterified fatty acids of lake sturgeon (*Acipenser fulvescens*) in the vicinity of hydroelectric facilities **2440–2447**

**Youson, J.H., J.A. Holmes, J.A. Guchardi, J.G. Seelye, R.E. Beaver, J.E. Gersmehl, S.A. Sower, and F.W.H. Beamish.** Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus* **2448–2456**

**Hutchings, J.A., R.A. Myers, and G.R. Lilly.** Geographic variation in the spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic **2457–2467**

**Hutchings, J.A., and R.A. Myers.** Effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic **2468–2474**

**Unwin, M.J., and D.H. Lucas.** Scale characteristics of wild and hatchery chinook salmon (*Oncorhynchus tshawytscha*) in the Rakaia River, New Zealand, and their use in stock identification **2475–2484**

**Xu, X., and R.B. Millar.** Estimation of trap selectivity for male snow crab (*Chionoecetes opilio*) using the SELECT modeling approach with unequal sampling effort **2485–2490**

**Salvadó, C.A.M.** Population field theory with applications to tag analysis and fishery modeling: the empirical Green function **2491–2512**

*(Continued inside back)*

CJFSDX50(11) 2293–2529 (1993)
ISSN 0706-652X

Canada

Typeset and printed in Canada by /
Composé et imprimé au Canada par
The Lowe-Martin Group: Runge Press Division

0706-652X(199311)50:11;1-8